ФИЗИОЛОГИЯ РАСТЕНИЙ

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A Translation Of

PLANT PHYSIOLOGY

(FIZIOLOGIIA RASTENII)

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THE ROLE OF PLASMODESMATA IN THE LOSS OF GERMINATION ABILITY OF COFFEE (COFFEA ROBUSTA LINN.) SEEDS

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More than three-quarters of a century have passed since plasmodesmata were discovered in the plant kingdom by Goroschankin [1] and Tangl [2]. During this time our knowledge of plasmodesmata has greatly expanded, nevertheless it should not be considered that plasmodesmata have received adequate attention since the volume of literature concerning them is comparatively small, as is evident from the latest review of this problem by Meeuse [3].

At the present time the protoplasmic nature of plasmodesmata is firmly established. Strugger [4–6] recently obtained some especially clear results. By making use of a new method for obtaining thin sections for the electron microscope and using selective staining with uranium acetate, he demonstrated the protoplasmic nature of plasmodesmata in the meristematic cells of the common onion root, with the electron microscope. The investigations in the laboratory at the Peking Agricultural Academy by Prof. Lou Cheng-hua and his co-workers [7] established the translocation of protoplasm from one cell to another in garlic, and also confirmed the early observations by Miehe [8] concerning the possibility of nuclear migration from cell to cell. This all points to the presence of a close protoplasmic relationship between the cells. At the present time a twofold function is attributed to the plasmodesmata. They are seen primarily as pathways along which nutrient substances are translocated from cell to cell, and secondarily as organs over which irritations are transmitted from one cell to another [3]. Mühldorf [9] attached special significance to this second function. In their investigations Genkel' and Oknina [10] and Oknina [11] showed that during dormancy the plasmodesmata were drawn into the cells, and the protoplasm of the resting cells remained separate. Similar results were obtained not only with dormant plants, but also with dormant seeds [10]. It should be noted that the process of protoplasm isolation, under the name of plasmolysis, was described by foreign authors [13, 14], however they did not comprehend the significance of the phenomenon which they disclosed and identified it with plasmolysis.

Sitnikova [15] demonstrated the presence of plasmodesmata in poplar aspen and willow seeds which rapidly lose their ability to germinate. According to the data, poplar, aspen and willow seeds rapidly lose their ability to germinate because of desiccation and the subsequent disruption of the plasmodesmata. In these experiments the seeds from one species of willow, Salix pentandra, had a true dormant period, the protoplasm in the cells was isolated, and there were no plasmodesmata. Poptsov and Buch [16] performed their experiments with two species of willow. They used Salix pentandra which has a clearly defined dormant period, and Salix nigricans whose seeds germinate without any dormant period. The seeds were kept in a desiccator under saturated salt solutions. As the result of these experiments the authors concluded that in Salix nigricans the lower the surrounding temperature the longer the seeds retained their germinating ability. The experiments were done at different degrees of relative humidity. At room temperature the most favorable relative humidity was 10%; at a temperature of 6–8° the most favorable relative humidity was 33%. After several months at these conditions there was a certain percentage of seed germination.

On the basis of our own experiments, we reject the explanation given by Sitnikova [15]. We feel that the authors did not verify the condition of the plasmodesmata and the isolation of the individual protoplasts, and

it is uncertain whether their report is correct. It is more likely that in their experiments the ability to germinate was retained in those cases where rapid desiccation of the seed coats occurred more quickly. Apparently during such treatment a layer impermeable to water was formed from the dry tissues on the surface of the seeds, and because of this the seeds retained their moisture and ability to germinate for a long time; the plasmodesmata; were undisturbed.

Finding ourselves on Hainan Island in tropical China during the end of December and the beginning of January, we decided to make use of the circumstances to become familiar with the dormancy period of tropical plants. As is known, most of the tropical plants have an extremely short period of dormancy. It was, therefore, extremely interesting to investigate whether the separation of protoplasm occurs in dormant tropical plants.

Unfortunately, because of our short stay on Hainan we were unable to carry out our investigation with woody species which were in a state of dormancy, and limited our study to the dormancy of seeds. From the literature it is known that the seeds of many of the tropical plants rapidly lose their ability to germinate, and some of them, like some of the Rhizophoraceae, have no dormant state and germinate immediately on the mother plant. Hevea seeds, which retain their ability to germinate for two weeks, as well as coffee seeds, belong to the group of seeds which rapidly lose their ability to germinate. This was also verified by the director of the state farm of tropical plants at Hsi Lung on Hainan Island by Comrade Liu. According to his data coffee seeds retain their ability to germinate for a month.

Comrade Liu very kindly gave us some germinating coffee seeds and reported that they germinate best at a temperature of 32°; we wish to express our appreciation to him. Our experiments and observations were done at the Institute of Plant Physiology, Shanghai Academy of Science, China; we are deeply grateful to the directors of the Institute, Professors Lo Tsung-lo and Ying Hung-ch'ang.

We stained the plasmodesmata of coffee seeds (Coffea robusta Linn.) according to Meyers method, as it is described in method guides [17]. In order to see the plasmodesmata we used a phase-contrast microscope. Sections were made from dry seeds, but in some of the experiments the seeds were soaked in water at room temperature for 24 hours. Sections 10 and 15 μ thick were made with a freezing microtome. The presence of plasmodesmota in the endosperm as well as in the tissue of the embryo was investigated. Figure 1 is a schematic drawing of stained plasmodesmata in endosperm cells of coffee seeds. The cell walls in the endosperm were extensively perforated with plasmodesmata. A similar picture is given in Fig. 2; this was made with a phase-contrast microscope. Plasmodesmata are clearly visible in the cell walls of the embryo in phase contrast (Fig. 3).

It is evident from the figures given that protoplasmic separation occur in coffee seeds, as was expected, but we were unable to detect a state of dormancy. Coffee seeds, as well as seeds from other species which rapidly lose their ability to germinate, did not go into a state of dormancy and quickly lose their power to germinate during desiccation because of the breaking and disruption of the plasmodesmata. In order to demonstrate the presence of favorable phenomena, we exposed the coffee seeds to a temperature of 40° for two and four days. As one can see from Figs. 4 and 5, high temperature brought about a distinct disruption in the condition of the plasmodesmata. The plasmodesmata seemed to be withdrawn from the wall into the cavity of the cell, and in most cases did not penetrate the entire wall as normally, but penetrated through only one-third or one-half of the thickness. In Fig. 5, made at high magnification, one can see that a small percentage of the plasmodesmata penetrated the entire thickness of the wall. Furthermore, the small thickening on the ends of the plasmodesmata are also very clearly visible here; in our opinion these represent a pathological formation.

One should note that it is much more difficult to observe injury to the plasmodesmata with the phase-contrast microscope than it is after they are stained.

In order to ascertain the effect of desiccation of the seeds as well as that of temperature, we kept the coffee seeds in a desiccator with sulfuric acid (1 : 1 dilution) for four days. As one can see from Fig. 6, after the seeds had been kept in the desiccator, the same kind of disruption of the plasmodesmata in the embryo cells was observed as that after exposure to a temperature of 40°.

At the same time that we investigated the presence of plasmodesmata, we also checked the germination of the coffee seeds. The experiments concerning germination of the seeds were done primarily on filter paper. One experiment was begun January 21, 1958 at a temperature of 35°. After eight days 60% of the seeds had germinated. In the second experiment, done at a temperature of 32° (January 25, 1958), 80% of the seeds had

germinated after nine days. In addition, two experiments concerning germination were set up in sand at a temperature of 32° and 25°. The experiments were started at the same time on February 9, 1958. After 10 days, 40% of the seeds in each experiment had germinated. As one can see, the germinating capacity of the seeds had dropped gradually; this experiment had been started one and one-half months after the seeds were obtained. In the experiment which was begun after 4 days of drying at a temperature of 40° (January 1, 1958) no germination was observed by February 8th. On February 4th coffee seeds which had been kept in a desiccator for four days were placed on moist filter paper. No germination was detected in this experiment either.

Fig. 1. Plasmodesmata in the endosperm of coffee seeds. Magnif. 10 x 40; Meyer's staining procedure.

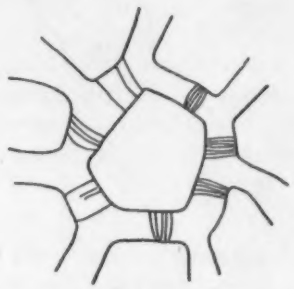

Fig. 2. Plasmodesmata in the endosperm of coffee seeds. Magnif. 12 x 45; phase-contrast microscope.

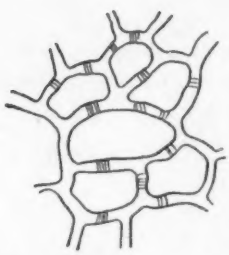

Fig. 3. Plasmodesmata in embryo cells of coffee seeds. Magnif. 12 x 45; Reichert's phase-contrast microscope; seeds previously soaked in water for 24 hr.

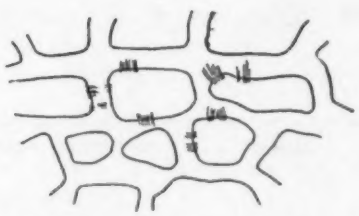

Fig. 4. Plasmodesmata in the endosperm cells of coffee seeds after drying in an oven at 40°C for 2 days. Magnif. 10 x 40; Meyer's staining procedure.

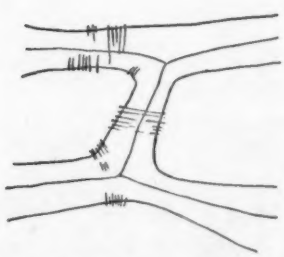

Fig. 5. Plasmodesmata in the endosperm cells of coffee seeds after drying in a desiccator (H_2SO_4 : H_2O 1:1) for four days. Magnif. 10 x 90(immersion); Meyer's staining procedure.

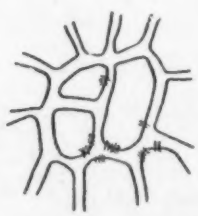

Fig. 6. Breaking of the plasmodesmata in the embryo cells of coffee seeds after drying in a desiccator (H_2SO_4 : H_2O 1:1) for four days. Magnif. 10 x 40 (immersion); Meyer's staining procedure.

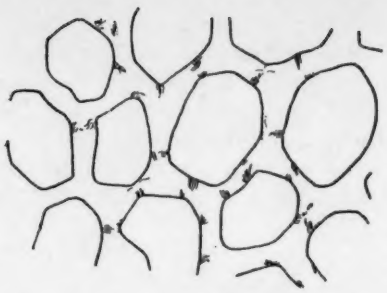

Fig. 7. Plasmodesmata in the endosperm, disrupted in nongerminating coffee seeds. Magnif. 100 × 10; oil immersion; Zeiss microscope.

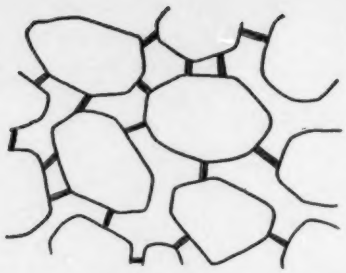

Fig. 8. Plasmodesmata in the endosperm of germinated coffee seeds (maintained in normal form). Magnif. 100 × 10; oil immersion; Zeiss microscope.

In March a germination experiment was set up in Moscow. The seeds were placed in quartz sand to germinate. Only one seed sprouted. The plasmodesmata in the endosperm of the nongerminated seeds were disrupted to a considerable degree (Fig. 7), whereas at the same time they remained normal in the germinated seed (Fig. 8).

From this we conclude that the disruption of the protoplasm and the plasmodesmata during desiccation of the seed results in the loss of the ability to germinate.

SUMMARY

Coffee (Coffea robusta Linn.) seeds, as well as seeds of other plants which rapidly lose their ability to germinate, do not have a dormant condition. Separation of protoplasm was not observed in such seeds which contain plasmodesmata in the endosperm cells as well as in the embryo cells.

The rapid loss of the germination ability in coffee seeds is connected with the deformation of the plasmodesmata, this occurs during drying of the seeds.

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• In Russian.

A STUDY OF THE FORMATION AND TRANSFORMATION OF CATECHINS IN TEA LEAVES USING $C^{14}O_2$

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The biosynthesis of various polyphenols and their role in the metabolism of plants has been studied very little. This can be explained primarily by the difficulty in isolating and identifying the composition of such a complex mixture of closely related compounds which usually occur in the polyphenol complex of plants. Until recently these difficulties were insurmountable; the development of the chromatographic method provided a means for solving similar problems. Isotopes are also very helpful in studying the mechanism of polyphenol formation [1-3].

A study concerning the formation and transformation of polyphenols has been in progress for several years in our laboratory [4-7]; tea leaves which have a special metabolism directed toward the synthesis and accumulation of catechins were used as experimental material.

A vigorous formation of catechins from stored substances has been observed in sprouting tea seeds [6, 8], at that time the simplest representative of this group of compounds, Lepicatechin, was synthesized primarily.

In a recently published paper [9] one of us showed that during photosynthesis catechin synthesis in the tea plant occurred mainly in the young three-leaved shoots, and a considerably smaller amount was synthesized at a slower rate in the mature leaves.

In the present investigation using $C^{14}O_2$, comparative observations were made on the behavior of sugars and the successive formation of catechins and their gallic esters in the young tea shoots. The experiments showed that catechins could be synthesized either during photosynthesis (in the light), or even in the dark at the expense of the sugars.

METHODS

Young three-leaved tea shoots fleshy (75-100 g) were placed in a round 6 liter flask provided with a three-holed rubber stopper. A dropping funnel was inserted in one of the holes.

The tube above the funnel contained 2 mc C^{14} in the form of $BaC^{14}O_3$, and the appropriate amount of $BaCO_3$ to obtain a 0.7-0.8% concentration of carbonic acid in the flask. The other two holes in the stopper were used later to pass air through the flask.

The flask was very slightly evacuated, and an excess of 10% H_2SO_4 was added drop by drop through the funnel into the test tube with $BaCO_3$. The flask which was protected from the direct rays of the sun with a light screen was placed outside, and the contents were carefully agitated by slowly rotating the flask. After one or two hours the carbon dioxide remaining in the flask was removed. Some of the shoots (15-20) were fixed with steam, and the remaining ones which were under conditions excluding the possibility of photosynthesis were placed between sheets of moist filter paper and fixed with a killing solution after appropriate time intervals.

The pulverized dry material (2g) was extracted twice with boiling 80% ethyl alcohol. The combined extracts were evaporated in a vacuum to 30-40 ml, the extract was filterd, and the filtrate was shaken with

chloroform, as described previously [10] (in order to remove the caffeins), and then the catechins were extracted with ethyl acetate.

In order to determine the sugars the water residue was evaporated under vacuum and 20 ml of the solution obtained was passed through a column (1.2 × 18 cm) with a cation carrier KY-2 in the H^+-form and an anion carrier PE-9 in the OH^- form. The eluate which drained from the column (~ 70ml) with the anion carrier and had an entirely neutral reaction was dried to a small volume (2-4ml) under vacuum and was placed on chromatographic paper.

A standard mixture of n-butanol-CH_3COOH-H_2O (4: 1 : 5) was used as a solvent; the chromatograms were checked with a face-recording densitometer and were developed with naphtharesorcinol, m-anisidine, aniline phthalate, or urea.

After they were eluted from the chormatograms, the sugars were determined by the anthranone method [14]; aliquots were used to check the activity on a face recorder.

In order to determine the catechins the combined ethyl acetate extracts were washed with a small amount of distilled water, dried with anhydrous $MgSO_4$ or Na_2SO_4, and after evaporation under vacuum were placed on chromatographic paper. Quantitative determinations were made using the method developed earlier [10].

Soluble Carbohydrates (Sugars)

Four main spots were observed on all the chromatograms; according to distribution these corresponded to fructose, glucose, sucrose and raffinose. In addition, one more spot with a low R_f value appeared on many of the chromatograms; this apparently corresponded to some sort of gluco-fructosan, or stachyose. These results agree with Torii's and Kanadzava's data [11], in which the presence of glucose, fructose, sucrose, stachyose, and apparently raffinose was disclosed in the leaves of a Japanese variety of tea. Cartwright and Roberts [12] also obtained closely related results which showed the presence of glucose, fructose, sucrose, ribose and possibly raffinose in Assam black tea. Hence, the idea expressed earlier concerning the presence of maltose in tea leaves [13] is apparently incorrect.

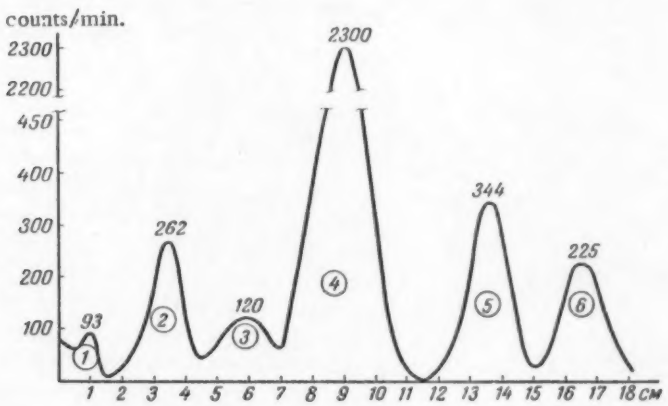


Fig. 1. A curve representing the distribution of radioactivity on sugar chromatograms in three-year-old tea shoots.
Exposure to $C^{14}O_2$ – 2 hours; activity checked on a face recording densitometer; 1) stachyose; 2) raffinose; 3) inositol (?); 4) sucrose; 5) glucose; 6) fructose.

Fig. 1 represents a curve of the distribution of the radioactivity of sugars in one of the chromatograms we obtained. The spot with the maximum at 6 cm did not develop as a sugar with the reagents, and apparently belonged to the meso-inositols.

The radioactivity of only four of the principal sugars – fructose, glucose, sucrose and raffinose – was studied in greater detail.

After young tea shoots had been exposed to light in the presence of $C^{14}O_2$ for 2 hours, the radioactivity in the sugars was distributed as follows (Table 1).

As we can see from Table 1, under these conditions more than 80% of the radioactivity of the total sugars was due to sucrose. Fructose and glucose were present in the tea shoots in considerably smaller amounts than sucrose, and their specific radioactivity was lower than that of sucrose.

TABLE 1

Distribution of Radioactivity in the Sugar Fraction after a Two-Hour Exposure of the Tea Shoots to Light in an Atmosphere with $C^{14}O_2$

Sugar	Amount in 1g dry weight of shoot (in mg)	Specific radioactivity in counts/min/mg dry wt.	Total radioactivity in counts/min/mg dry wt.	Radioactivity in % of total
Fructose	0.72	205 000	147 600	6.0
Glucose	1.0	219 000	219 000	9.0
Sucrose	7.2	278 000	2 001 600	81.8
Raffinose	1.8	43 300	77 900	3.2

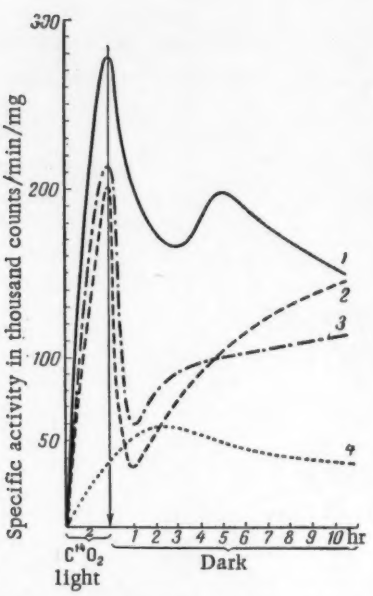


Fig. 2. The change in specific radioactivity of the sugars when tea shoots were kept in an atmosphere without $C^{14}O_2$ at conditions prohibiting photosynthesis (in counts/min/mg). 1) sucrose; 2) fructose; 3) glucose; 4) raffinose.

After 2 hours raffinose was labeled even more weakly, and only 3.2% of the total radioactivity of the sugars was due to it.

On the basis of results obtained, one can consider that in the tea leaves, as well as throughout the plant, not synthesizing tannins, the first free sugar which appears during photosynthesis is sucrose [15, 16]. Other sugars are formed mainly as a secondary reaction, i.e., as the result of the further transformation of sucrose.

The correctness of such a conclusion also ensues from the results of experiments concerning the behavior of sugars after the tea shoots were removed from an atmosphere with $C^{14}O_2$.

The curves presented in Fig. 2 for the specific radioactivity of the sugars indicate the complex processes occurring in the tea leaves.

At first, when the shoots were exposed to light in an atmosphere with $C^{14}O_2$ for 2 hours there was a vigorous accumulation of labeled sugars. After the removal of $C^{14}O_2$ and the discontinuation of photosynthesis, within an hour the specific radioactivity of sucrose, and especially that of glucose and fructose, dropped sharply.

At first glance the decrease in specific radioactivity of the sugars in the dark contradicts the basic principle of the utilization of isotopes since it indicates a more rapid consumption of labeled molecules as compared with unlabeled ones. One might partially assume that the decrease in specific radioactivity of the sugars occurred because of their "dilution" with nonradioactive sugars which resulted from the dissociation of starch. However, it is known that starch, as well as sucrose, is very strongly radioactive during photosynthesis in an atmosphere with $C^{14}O_2$ [15, 16], consequently the marked "dilution" of the specific radioactivity of sugars by products of starch hydrolysis is not very likely. Therefore, we must assume that under the conditions of our experiments the labeled sugars were actually consumed more rapidly. However, this was apparently not caused by the difference in chemical structure of the labeled and nonlabeled sugars, but by the spatial localization of the photosynthetic products within the cell. For example, if sugar or starch appears in the chloroplasts during photosynthesis, it is usually concentrated up to some limit. It is true that the diffusion of soluble products of photosynthesis into the protoplasm occurs from here, however this process apparently does not keep up with their new formation. If this is

true, then the concentration of labeled sugars (their specific radioactivity) should be higher in chloroplasts which have been photosynthesizing for some time in $C^{14}O_2$ than in the remaining parts of the cell.

Furthermore, it is known that plastids, and therefore chloroplasts, are the site of the concentration of many enzymes [17]; this makes rapid transformation of materials in them possible. It is therefore completely understandable that after the discontinuation of photosynthesis, especially in the chloroplasts, the photosynthates concentrated in them were subjected to the greatest rate of change, and depended on respiration for the secondary transformation. All this leads to the conclusion that during an estimate of the specific radioactivity of the sugars for all the tissue, without counting the localization of photosynthetic products within the cells themselves, there appeared to be selectivity in the utilization of labeled molecules.

TABLE 2
Sugar and Starch Content of Young Three-Leaved Tea Shoots
(Average data of two observations)

Carbohydrates	Content, in mg per g dry weight
Monosaccharides	2.7
Sucrose	8.3
Starch	4.7

The curve representing the behavior of sucrose is of greatest interest in the problem under consideration inasmuch as most of the radioactivity was concentrated in this fraction of the sugars. As one can see in Fig. 2, after $C_{14}CO_2$ was removed the specific radioactivity of sucrose began to decrease rapidly; this is indicative of its consumption in the chloroplasts.

After the tea shoots had been kept in the dark without $C^{14}O_2$ for 5 hours, the general decrease in specific radioactivity of sucrose changed temporarily to an increase, after that the specific radioactivity continued to decrease again. It is quite possible that this increase was associated with a mobilization of the assimilated starch contained in the chloroplasts; according to the data of some authors [15, 16, 18] the introduction of radioactivity into this portion was quite significant. Hence one can conclude that during comparatively short light exposure the deposition of starch in the chloroplasts occurs primarily by superposition over already existing starch granules. Therefore the outer layers of starch granules should be distinguishable by their especially high specific radioactivity. If we also take into account the ease of the reciprocal transformation of starch and sucrose in plants [19-21], it is easy to conclude that the beginning of the hydrolysis of such radioactive starch can result in the increase of specific radioactivity of sucrose. In the present investigation there was no direct measurement made of the radioactivity of starch. However, analyses of three-year-old tea shoots (see Table 2) revealed the presence of a notable amount of starch in them together with the monosaccharides and sucrose; it was sufficiently high so that during its hydrolysis there could be an increase in the specific radioactivity of sucrose which apparently was observed in this experiment.

Later, in the interval between 5 and 11 hours (see Fig. 2), the specific radioactivity of sucrose decreased again, and by the end of the experiment it had dropped almost twice that of original value. As we have already noted, the principal consumption of radioactive sucrose (which results in the decrease of its specific radioactivity) can be explained by its concentration in the chloroplasts, where sugar has been found to be transformed more rapidly than in other parts of the cell because of the vigorous enzymatic systems.

The specific radioactivity of monosaccharides changed quite differently. Immediately following the sharp decline in radioactivity during the first hour after the shoots were removed from an atmosphere with $C^{14}O_2$ the specific radioactivity of glucose as well as fructose began to rise again; this was apparently due to a secondary formation of monosaccharides from compounds with a higher radioactivity (probably from sucrose and starch), and the gradual movement of hexoses by diffusion from the sphere of vigorous activity (i.e., from the chroroplasts into the protoplasm). In comparison with the other sugars the fluctuation in the activity of raffinose was considerably less pronounced; this is evidence of the comparative inertness of this trisaccharide in the carbohydrate metabolism of tea shoots.

When we calculated the change in radioactivity of each sugar per gram dry weight of the tea shoot for the last 10 hours of the experiment (i.e. after the nature of the processes had been ascertained to a certain degree), the following results were obtained (Table 3).

TABLE 3.

The Change in Total Radioactivity of Sugars in Young Tea Shoots Following Their Removal From an Atmosphere with $C^{14}O_2$ (between the first and eleventh hour of the experiment) (in counts/min/1 g dry weight of shoots)

Sugar	Exposure		Change of radioactivity in 10 hr of darkness	
	2 hr in $C^{14}O_2$ followed by 1 hr in the dark without $C^{14}O_2$	2 hr in $C^{14}O_2$ followed by 11 hr in the dark without $C^{14}O_2$		
Fructose	24 440	105 870	+ 81 100	+135 400
Glucose	58 700	113 000	+ 54 300	
Sucrose	1 497 600	849 300	−648 300	−668 200
Raffinose	94 950	75 050	− 19 900	
Total Sugars	1 672 020	1 143 220	−532 100	

From Table 3 it is evident that labeled sucrose and raffinose were used in the tea shoots during the last 10 hours of the experiment, and that there was an accumulation of labeled glucose and fructose. However, these two processes did not compensate for each other. Only about 20% of the radioactivity lost from sucrose and raffinose appeared in glucose (8.1%) and fructose (12.3%). The remaining radioactivity was apparently utilized in respiration or, as we will show below, in the synthesis of catechins.

Catechins

Together with the study of the radioactivity of sugars, we also determined the radioactivity of catechins in the same material. In order to get a more adequate representation of the sequence of their formation in addition to the two-hour exposure to $C^{14}O_2$, part of the material was kept in an atmosphere with radioactive $C^{14}O_2$ for ust 1 hour, and was fixed immediately after this. Fig. 3 shows the change in specific radioactivity of the catechins when the young tea shoots were kept in the light for 1 or 2 hours in an atmosphere with $C^{14}O_2$ and then in the dark without $C^{14}O_2$.

TABLE 4

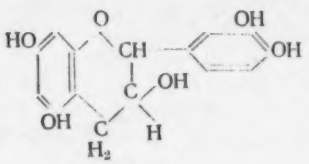
Change in Total Radioactivity of the Catechins in Tea Shoots in the Period Between 3 and 11 Hours after Removal of $C^{14}O_2$

Catechins	Amount in 1 g dry weight of shoot, in mg	Total radioactivity in counts/min/1 g dry weight		Change in 8 hr
		After 3 hr	After 11 hr	
l-epicatechin	7.3	93 440	71 100	− 22 340
l-epigallocatechin	29.4	723 240	297 560	−425 680
d, l-gallocatechin	4.5	65 700	49 500	− 16 200
l-epicatechingallate	15.3	114 140	134 030	+ 19 890
l-epigallocatechingallate	68.2	453 530	606 980	+153 450
Total catechins	124.7	1 450 050	1 159 170	−290 880

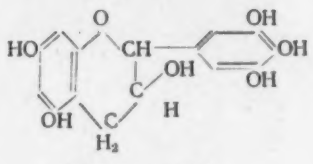
A study of the curves in Fig. 3 reveals that the catechins were already radioactive after the tea shoots were exposed to $C^{14}O_2$ for 1 hour. Judging by the comparatively high radioactivity after this time of exposure, their synthesis must have begun immediately after the synthesis of sugars, and may have occurred at the same time. The continued rise in specific radioactivity of the catechins during the continued exposure of the shoots to an atmosphere with $C^{14}O_2$ up to 2 hours also indicates the close relationship between the synthesis of catechins and photosynthesis. However, Fig. 3 also shows that after $C^{14}O_2$ was removed, and the tea shoots were placed under conditions where photosynthesis did not occur, the synthesis of labeled catechins still continued for 2-3 hours, whereas at the same time the radioactivity of the sugars began to decrease, as we showed above (see Fig. 2). This was the basis for considering that the catechins in the tea leaves can be synthesized secondarily from sugars or from products of their breakdown.

It is possible that the synthesis of catechins is accomplished more readily from intermediate products of photosynthesis which are high in energy than from the hydrolysis of such stable compounds as sugars. At any rate, the more rapid introduction of C^{14} into the catechins during photosynthesis rather than during their secondary synthesis from labeled sugars is indicative of this.

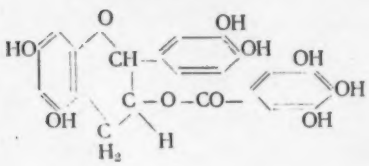
Apparently the increase in the specific radioactivity of the catehcins (especially the simple catechins, i.e. L-epicatechin and L-epigallocatechin) during the first hours after darkening and removal of $C^{14}O_2$ can be explained expressly by their synthesis from sugars which had a higher radioactivity.



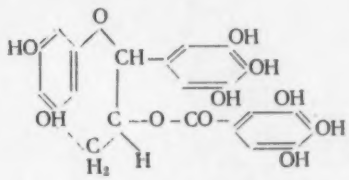
epicatechin



epigallocatechin



epicatechingallate



epigallocatechingallate

As we have already mentioned above, in contrast to the sugars, the specific radioactivity of catechins in tea shoots continued to increase even after the removal of $C^{14}O_2$ when carbohydrates were undoubtedly the primary source for the increase in radioactivity of the catechins. The pathway of carbohydrate "phenolization" can be represented in various ways. For example, during certain transformations of carbohydrates sedoheptulose-1, 7-diphosphate appears; this is one of the primary substances in the synthesis of shikimic acid [22,23] and later the pyrocatechin or pyrogallate nucleus of the flavone molecule [1] and enters into the composition of lignin from aromatic aldehydes [24]. During the glycolytic dissociation of carbohydrates activation of the acetyl residue, which serves as the base for the formation of the phloroglucin nucleus of anthocyanins, also occurs [3].

Calculations showed that in the experiment under consideration the total radioactivity of the catechins in 1 g dry weight of shoots 1 hour after the removal of $C^{14}O_2$ consisted of 1,366,000 counts/min, whereas 3 hours after the removal of $C^{14}O_2$ it was 1,450,000 counts/min. Hence, during these 2 hours the radioactivity of the total catechins increased by 84,000 counts/min per 1 g dry weight. If we compare this figure with the decrease in radioactivity of the sugars (see Table 3), assuming that the activity of the sugars changed equally during the last 10 hr of the experiment, it is evident that the loss of radioactivity of the total sugars in 2 hr consisted of 106,600 counts/min per 1 g dry weight of the shoots. From this we can conlude that in tea shoots most of the radioactivity of the sugars was lost in the synthesis of catechins (84,000 counts/min in 2 hr.), and a smaller portion (22,600 counts/min) was dispersed during respiration, the synthesis of cellulose, and other processes.

The increase in radioactivity of the total catechins continued for 3 hours after the tea shoots were removed from an atmosphere containing $C^{14}O_2$. Later the overall radioactivity of the total catechins began to decrease, while the change in specific radioactivity of the specific catechins varied. At the time the specific radioactivity of the simple catechins (L-epicatechin, L-epigallocatechin, and D,L-gallocatechin*) decreased sharply, it continued to increase in general in the gallolyl-containing catechins (L-epicatechingallate and L-epigallocalingalate) until the end of the experiment.

The variation in the behavior of simple catechins and gallolyl-containing catechins after the removal of $C^{14}O_2$ showed up very clearly during the change in direction of the magnitude of the specific activity toward the

* In Fig. 3 and 4 the curves for D,L-gallocatechin are not given for simplification.

original value, i.e. to the specific activity of the given catechin measured immediately after the termination of 2 hours of photosynthesis in an atmosphere with $C^{14}O_2$ (Fig. 4).

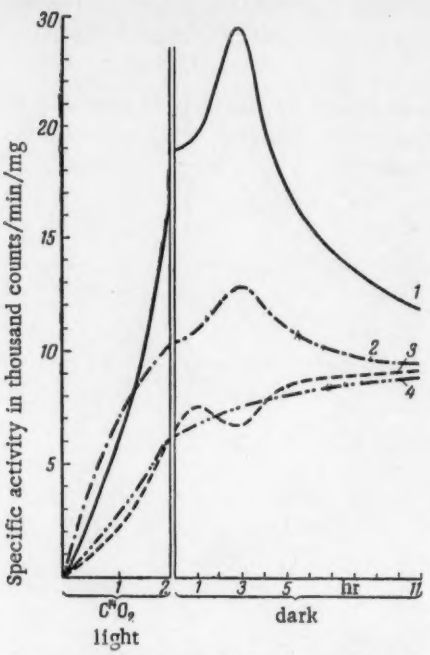


Fig. 3. Change in specific radioactivity of the catechins during the exposure of tea shoots to an atmosphere with $C^{14}O_2$ in the light, followed by conditions not permitting photosynthesis and without $C^{14}O_2$ (in counts/min/mg).
1) L-epigallocatechin; 2) L-epicatechin; 3) L-epigallocatechingallate; 4) L-epicatechingallate.

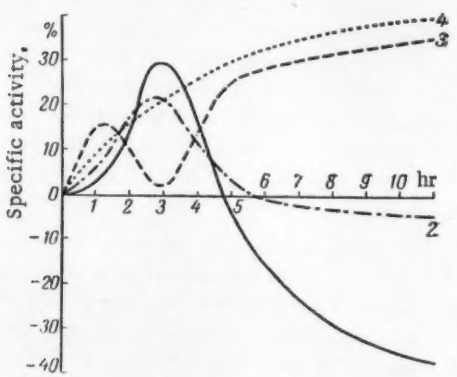


Fig. 4. Change in specific activity of catechins when tea shoots were kept in an atmosphere without $C^{14}O_2$ (in % of initial value).
1) L-epigallocatechin; 2) L-epicatechin; 3) L-epigallocatechingallate; 4) L-epicatechingallate.

The mirror-like behavior of simple and gallolyl-containining catechins led us to conclude that the synthesis of gallates occurs from simple catechins and is a secondary process that occurs later; this is also indicated by the distribution of catechins after a one-hour exposure to light in an atmosphere with $C^{14}O_2$ (Fig. 3). Gallic acid which is necessary for the formation of gallolyl-containing catechins exists in the free state [25] and is changed enzymatically to catechins either immediately or via intermediate acceptors.

On the basis of the significant decrease in specific radioactivity of simple catechins 3-11 hours after the beginning of exposure to an atmosphere without $C^{14}O_2$ (Fig. 3 and 4), one can conclude that the labeled catechins resemble sucrose and the monosaccharides (see above) in being unequally distributed within the cell, and becoming involved in metabolic processes immediately because of their proximity to corresponding enzyme systems in the plastids.

It is evident from Table 4 that during the time interval between 3 and 11 hours after the removal of $C^{14}O_2$ the decrease in total radioactivity of the simple catechins was not completely compensated by the increase in radioactivity of the gallates.

During this period (8 hours) the decrease in radioactivity in the catechin fraction was 290,880 counts / min per 1 g dry weight; this consisted of about 20% of the amount of total radioactivity of the catechins 3 hours after the tea shoots were removed from an atmosphere with $C^{14}O_2$. The decrease in the radioactivity of the catechin fraction can hardly be explained by the association of catechins with proteins and their subsequent transformation into an insoluble form, since in this case the larger molecules of the gallic esters of the catechins would have been involved first in this process, and would have decreased their total radioactivity more rapidly than the others.

Therefore, despite the opinion of others, it can be assumed that in higher plants catechins do not occur as end-products of metabolism, but are capable of a further deep-seated transformation, similar to that which occurs with polyphenols in various microorganisms [26,27].

We wish to note that during expeditionary experiments in Georgia we obtained assistance from I. D. Gamkrelidz, the director of the Institute of Tea and Subtropical Plants; the senior scientific co-worker of the Institute, A. I. Uturgaur; and the senior scientific co-worker at the Institute of Tea Cultivation, M. N. Shavishvil.

SUMMARY

The following sugars were detected in young three-leafed shoots of the Georgian variety of tea plants by the paper chromotography technique: sucrose (7.2 mg/gr of dry weight), glucose (1.0 mg), fructose (0.72 mg) and two other more complex sugars corresponding to raffinose by their R_f values (1.8 mg.) and stachyose (very insignificant amounts).

After the tea shoots were exposed to light for 2 hours in an atmosphere containing $C^{14}O_2$ the greater part of radioactivity of the sugar fraction was found in sucrose (up to 82%) whereas in glucose and fructose together it constituted only 15%. Raffinose was very weakly labeled after the 2 hour period.

When the assimilation of $C^{14}O_2$ stopped the specific and total radioactivity of sugars in the leaves of the tea plant underwent considerable changes (Fig. 2 and Table 3). This can be explained by rapid consumption of these substances, especially of sucrose.

When photosynthesis occurred in an atmosphere containing $C^{14}O_2$ radioactive catechin accumulated along with sugars during the first two hours. This shows that the formation of catechin is related to the products of photosynthesis. Simple catechins have the largest specific radioactivity under these conditions and their gallic esters are labeled to a smaller extent.

After $C^{14}O_2$ was removed and the light turned off the specific and total radioactivity of catechin in shoots of tea plants continued to increase over a period of three hours. (Fig. 3). This and the rapid consumption of labe labeled sugars indicates that secondary synthesis of catechins is possible. In this case as well as during photosynthesis the formation of simple (non galloyl-containing) catechins is more intense.

Three hours after $C^{14}O_2$ was removed and afterwards the specific and total radioactivity of simple catechins greatly diminished whereas the radioactivity of their gallic esters (Fig. 4 and Table 4) still continued to grow. This indicates the secondary nature of the processes of galloyl attachment.

A study of the changes of the total radioactivity of all sugars and catechins after removal of $C^{14}O_2$ permits one to conclude that the greater part of the sugars (approximately up to 80%) in the young shoots of the tea plant are utilized for this synthesis of catechins.

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THE UTILIZATION OF SOLAR RADIATION DURING PHOTOSYNTHESIS OF POTATO CROPS

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Solar radiation is one of the most important ecological factors for plants. Such an evaluation of this factor is dependent primarily on the fact that the principal process of plant nutrition, photosynthesis, occurs with solar radiation as the energy source. However, in the process of photosynthesis itself only 1-2%, and in special cases up to 5%, of the total energy absorbed by the leaves is bound or transformed into chemical energy. The remainder is transformed into heat, some of which (a comparatively small part) is lost into the surrounding medium by means of heat radiation, but most of it induces the evaporation of water by the leaves-transpiration.

Even in those instances when photosynthesis occurs with a theoretically minimum quantum requirement, namely, with 8-12 quanta of absorbed photosynthetically active light for one molecule of reduced CO_2, the "coefficient of efficiency" is only 23%. One einstein of visible light corresponds approximately to 50 kcal/mole quanta, and the energy of 8-12 quanta is close to 500 kcal. The amount of energy bound in the form of chemical energy in a gram mole of photosynthate (CH_2O) is 112 kcal; this corresponds to 23% of the absorbed energy. However this theoretical value can be obtained only under extremely favorable laboratory conditions. Actually under natural conditions the efficiency coefficient of photosynthesis using the energy of solar radiation absorbed by the leaves is several times lower than the 23% mentioned. This is due to the following factors: first, together with the photosynthetically active rays of visible light the leaves also absorb rays which are not active in this respect, for example infrared rays; this consists of about 45-50% of the energy of the total solar radiation, and up to 30% of that absorbed by the leaves.

Furthermore, under natural conditions photosynthesis is usually decreased by a whole series of such unfavorable conditions as insufficient moisture, poor nutrient supply, low CO_2 content of the air, restricted growth of the plants, and together with this, the restricted use of photosynthates, etc. Consequently, under natural conditions the actual photosynthesis observed is 5-10-20 times less than the rate theoretically possible.

In the meantime it is extremely important to note that aside from the low efficiency coefficient of photosynthesis for the energy absorbed by the leaves, the leaves cannot do their best in low light intensities. On the other hand, practically all the crop plants are heliophytes and require high light intensities for their normal growth and photosynthesis. This pertains especially to plantings as a whole where there is mutual shading of the leaves and where even a slight shielding of the sun with clouds induces a practically proportional decrease in the total photosynthesis of the leaves as Thomas and Hill [6], for example, have shown.

Hence, if we want to obtain high yields, it is essential to provide the plants with the best light conditions. However as a consequence of this the leaves absorb a great deal of radiant energy and much water is lost by transpiration or, if the amount of water available to the plants does not correspond with the amount of energy absorbed by the leaves of the plant, the plants become overheated.

These things account for the fact that by weight crops lose several hundred more times water during transpiration than they add as dry weight yield in the process of photosynthesis. The inherent contradictory biology of the plant and the problems of getting high yields is therefore quite a puzzle.

In the long run, in order to get high crop yields in the plantings we should strive for a uniform stand of plants, for uniform dimensions of the leaf blades, and possibly a better absorption of the radiant energy falling on them. Furthermore the plantings should not be excessively thick in order not to bring about excessive mutual shading of the leaves and impair conditions for photosynthesis; these frequently result in a decrease in the quality of the harvest, due to layering of the plants, to retardation of growth of the reproductive and storage organs, etc.

An important factor in obtaining high yields is the formation of sowings and plantings with an efficient structure, and especially with an efficient optimal growth of leaf blades [1]. However, plantings with an efficient structure and an optimal leaf surface which absorb a large quantity of radiant energy can be otained only with an adequate water supply and good nutrition.

Usually the amount of energy absorbed by plantings is so great that even in those zones where the yearly standards of precipitation correspond to 600-700 mm (the Moscow zone, for example), the plantings respond positively to additional watering. Some of the prerequisites for obtaining high yields are alike. As we see,they are based on data concerning the energy balance of the plantings.

In the investigations concerned with obtaining high yields we should ultimately strive to have the plantings absorb the very greatest amount of energy possible, to have the water supply of the plantings correspond to the amount of energy absorbed by them, and to have the mineral nutrition and other factors favorable for the highest efficiency coefficient of the absorbed energy in the process of photosynthesis and the lowest possible in transpiration [1, 2]. It is therefore clear that experimentation, a constant control, and a calculation of the energy balance of the crop should serve as the basis of a study of the theory and practice for obtaining high yields.

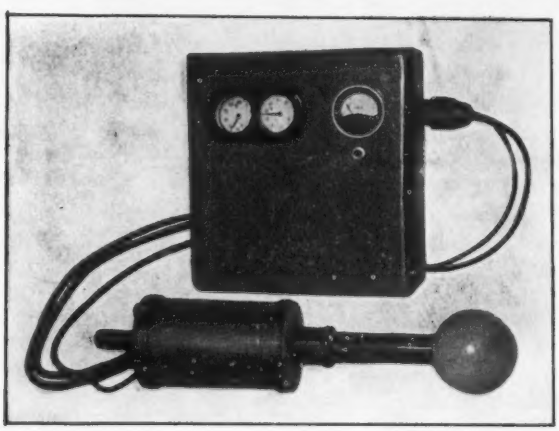

Fig. 1. External view of photointegrator

With this objective in mind in 1957 we carried out some investigations together with Prof. A. G. Lorkh on the experimental farm at the Scientific Experiment Institute of Potato Farming near Moscow..

The work was done on plantings of potatoes (with a planting design of 40 × 60 cm and 80 × 60 cm) and corn (sowing design 30 × 60 cm and 70 × 70 cm).

The object of our investigation was to find out the amount of energy absorbed by the plantings in relation to leaf area, sowing design, and also the type of plant.

A photointegrator (Fig. 1) with a cesium vacuum photoelement Ts V-3 and with an integrating design provided with a mechanical calculator which totaled the amount of radiant energy during the time of exposure served as a self-recording device; it was made in the Photosynthesis Laboratory at the Institute of Plant Physiology, AN SSSR, by V. P. Kornil'ev with the assistance of L. N. Bella and the authors. Instruments described by Middleton [7] served as prototypes of other similar photointegrators.

The presence of glass light filters (SZS-14 and Zh S-11) in these instruments provided for an unselective

sensitivity of the receiver in the region of photosynthetically active radiation (500-720 m μ).

Glass spheres which dispersed the light and permitted the measurement of the average amount of energy falling on the spheres were used to receive the radiant energy.

The selection of such receivers was associated with a whole series of special illumination features in grass stands. The leaves and stems receive light which falls on them at very different angles. This can be either direct sunlight, or light reflected from the sky, or reflected from plants that are constituents of the environment.

Spherical receivers absorb the light rays in a stand of grass normally falling on the surface at various angles and in this way decrease the inevitable errors which are obtained during similar measurements with flat light receivers.

The integrating design with a spherical receiver was also chosen because the use of a flat receiver with instantaneous readings which records only the direction or rate of radiation is impractical because the receiver may be under accidental circumstances at any time (flashing of the sun, shading by a shadow of the leaf, stem, etc.). Furthermore, single instantaneous recordings can be characterized by accidental circumstances at the given moment (direct sun light, shading by a cloud, etc). The integrating design makes it possible to calculate the sum of radiant energy for any sufficiently long time interval even when the conditions of radiation are not constant.

Two photointegrators were used for each calculation. One was placed below the plants and measured the total (S = A + B) energy falling on the plants (A) and that reflected from them (B). The second was placed in the lowest layer of leaves and measured the total (T = S + D) energy which passed through the depth of the planting (S) and was reflected from the soil (D).

The integrating design made it possible to consider the total amount of energy (dose) in the categories indicated above (S and T) for each hour during a period of several hours or during a whole day of observations.

The amount of energy absorbed by the plants (R) was calculated by the equation $R = (S-B)-(I-D)$, where $S-B=A$, i.e. the energy falling on the plant, and $T-D=S$, i.e. the energy passing through the depth of plants.

On the basis of several observations, with the help of Iu. D. Ianishevsky's reflection factor, it was established that the amount of energy reflected by the plants consisted of about 20% of that which had been recorded by the photointegrator placed above the plants.

Therefore, the amount of energy falling on the plants was found to be 80% of the energy recorded by the above mentioned photointegrator, i.e.

$$A = \frac{S \cdot 80}{100}$$

The value D (reflected from the soil under shelter of the plants) was often insignificant and was not calculated. Hence, in our calculations the amount of energy transmitted by the plants (S) was assumed to be equal to that recorded by the lower photoindegrator (T). Under this condition the amount of solar radiant energy absorbed (R) by the plants was finally calculated by the equation:

$$R = \frac{S \cdot 80 - T}{100}$$

First of all, the results we obtained made it possible to get an overall picture of the radiation process on the environs of the Potato Institute near Moscow in 1957. The system of light filters in our photointegrator made it possible for it to register only visible light, and at the same time photosynthetically active radiation, which was about 50-45% of the total radiant energy. Multiplying the values for the daily amount of radiant energy recorded by the photointegrator by the coefficient 2.2, we obtained the figure for the daily amount of total radiation.

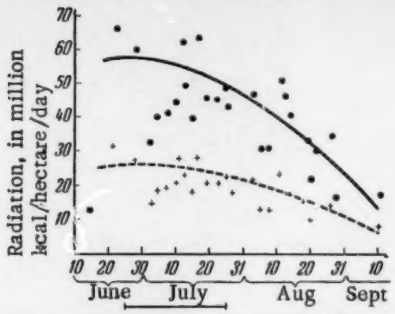

Fig. 2. The receipt of energy from solar radiation (million kcal/hectare in a day) during the growing period.
Dots and solid line – total radiation; crosses and broken line – photosynthetically active radiation.

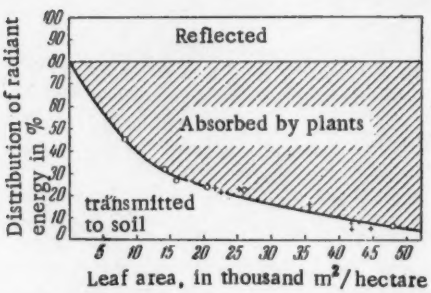

Fig. 3. The distribution of photosynthetically active radiation falling on the plants into that reflected, absorbed by the plants, and transmitted to the soil in relation to the leaf area of potato (+) and corn (o) plants.

These and other indices obtained in 1957 during the growing period are given in Fig. 2.

From the data in Fig. 2 it is evident that the daily amount of energy of total radiation near Moscow expressed in figures was from 50-60 million kcal/hectare in a day during the middle of the growing period, and 20 million kcal/hectare in a day at the end of the growing period. The corresponding amount of photosynthetically active radiation expressed in figures was 27-25 and 8-10 million kcal/hectare in a day. These values agree closely with data obtained by other investigators [3-5].

Moreover, using the method described above we obtained the percentage and the absolute value of the energy of photosynthetically active solar radiation absorbed by stands of various plants during various planting procedures. The data obtained from these observations are given in the table and in Fig. 3.

The figures in the table show that the percentage of photosynthetically active radiant energy absorbed by potato and corn plants was determined primarily by the leaf area, in spite of the sowing procedure. For example, when potato plants were distributed according to the design of 40 x 60 cm and 80 x 60 cm, the values for the solar energy absorbed coincided when the leaf areas reached the same value. However, the increase in leaf area and the percentage of energy absorbed in the planting with a 40 x 60 cm distribution occurred about two weeks earlier than the increase of these values in the planting with an 80 x 60 cm distribution.

On the basis of the determinations made concerning the percentage of solar energy absorbed in dense grass stands with a leaf area of from 20-25 to 42-48 thousand m^2/hectare, and assuming that the amount of energy reflected by the plants was about 20-25% according to reflection measurements by Iu. D. Ianishevski, we made a graph for the distribution of energy from solar radiation as that reflected, absorbed, and transmitted to the soil in stands of potato and corn in relation to leaf area (Fig. 3).

We had made some similar graphs earlier [1] on the basis of data in the literature and some calculations which at that time were not yet classified.

Comparing the graph made earlier with the present one, one can see that they agree quite well. These data show that during the period when the canopy of the plantings was becoming more compact and the leaf area increased to 35-40 thousand m^2/hectare the plants absorbed 60-75% of the energy in the photosynthetically active radiation, and that any further increase in leaf area brought about an insignificant increase in the amount of energy absorbed.

When the leaf area increased to 10 thousand m^2/hectare the energy absorbed by the plants increased by 40%, when the leaf area increased from 10 to 20 thousand m^2 hectare it increased by 16%, when it increased from 20 to 30 thousand m^2/hectare it increased by 8%, when it increased from 30 to 40 thousand m^2/hectare it increased by 6%, and when it increased from 40 to 50 thousand m^2/hectare it increased by only 4-5%.

This case is one example favoring the conclusion that a leaf area of 35-40 thousand m^2/hectare can be considered optimal for obtaining high yields, and an increase in this value is not very expedient, or else is associated with an especially great expenditure of water and minerals.

TABLE

The Amount of Photosynthetically Active Radiation (in % of that Falling) Absorbed by Potato and Corn Plants with Various Leaf Areas

Date of observations	Leaf area of planting in thousand m^2/hectare	Energy absorbed by plants in %	Date of observations	Leaf area of planting in thousand m^2/hectare	Energy absorbed by plants in %
Potatoes					
	Planted 40 × 60 cm			Planted 80 × 60 cm	
24 June	25.3	57.5	4 July	22.0	57.5—58.5
5 July	36.7	63.5—64.0			
25 July	42.0—45.0	74.0—75.0	16 Aug	42.5	73.0—74.0
Corn					
	Planted 30 × 60 cm			Planted 70 × 70 cm	
7 Aug	14.5	48.0	7 Aug	7.6	35.0
19 Aug	25.4	57.2	22 Aug	16.2	52.0
11 Sept	48.0	72.2--73.5	28 Aug	20.2	56.5

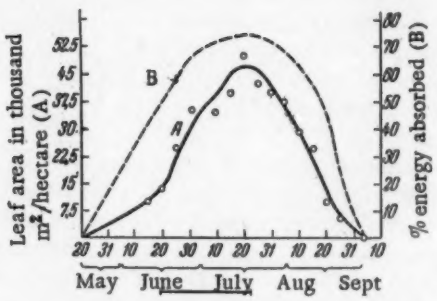

Fig. 4. The course of increase in leaf area (curve A) and the percentage of the energy of photosynthetically active radiation falling on the plants absorbed by the plants (curve B) in potato plantings in 1957.
Curve A — according to L. E. Strogonov's calculations; curve B — obtained on the basis of data in Fig. 3.

The ultimate object of our observations was to determine the absolute amount of energy absorbed by the plants and, by comparing this with the increase in dry weight of yield and the amount of energy accumulated, to determine the efficiency coefficient for solar radiation used in photosynthesis. This work was done on potato plantings with a general planting design of 60 × 40 cm. The increase in leaf area of the potato plantings on which the observations were made is given in Fig. 4 (curve A — determined by L. E. Strogonov). In this figure the given percentages of energy falling on the plants that were absorbed at various times of the growing period (curve B) depended on the size of leaf area.

From Fig. 4 it is evident that the vigorous increase in growth of leaf area was observed during the period from the middle of June until the end of July. After this the lower leaves began to turn yellow and their total production decreased, the shoots bent to the ground, and it became difficult to measure the radiation relations within the planting.

A significant decrease in the direct products of photosynthesis and the total daily increase in dry weight of the harvest occurred after the middle of July (Fig. 5). In connection with this, after the middle of July the general rate of dry weight increase of the harvest slowed down (Fig. 6, curve 1), even though there was still an intensive increase in the total harvest of tubers (Fig. 6, curve 3). However, during this time of the growing period the growth of the tubers occurred at the expense of a redistribution of metabolites and their intensive translocation from the leaves, whose weight dropped sharply during this period (Fig. 6, curve 2).

Hence, the period of most rapid and intensive photosynthesis (according to summary results), is the period of the greatest increase in total dry weight of the harvest was during the period from the middle of June until the end of July. We therefore centered our attention on this period to determine the efficiency coefficient for the use of radiant energy in photosynthesis.

In Figs. 2, 4-8, this period is marked with a dark line below the axis of the abscissa. It should be mentioned that within this period there was a period of cold rainy weather which lasted from June 23rd to 24th, during which the indices of growth and photosynthesis in potato were low. After June 25th warm weather, even though

variable, became established. The combination of favorable weather with good agricultural methods and fertilization applied by Prof. A. G. Lorkh resulted finally in a high daily increase in total dry weight which reached 200 kg/hectare in a day (Fig. 5), and in obtaining a high final yield of 83 centner/hectare of total dry weight (Fig. 6) and 430 centner/hectare of fresh tubers. It is true that the value for the direct products of photosynthesis was comparatively low (4-6 g/m^2) in a day, but this was compensated by the presence of a total leaf area which reached 35-40 thousand m^2/hectare and an absorption of 60-75% of the energy of physiologically active solar radiation falling on the plants.

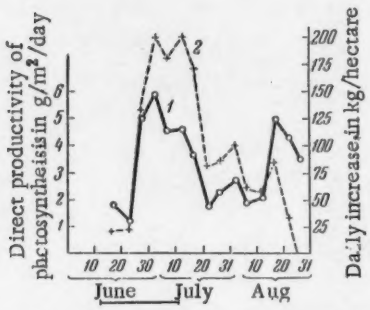

Fig. 5. A curve representing the direct products of photosynthesis (1) in g/m^2 of leaf area in a day, and the daily increase in total dry weight of the harvest (2) in kg/hectare in a day in potato plantings.
The data were obtained by A. G. Lorkh and L. E. Strogonov in 1957 and processed by the latter.

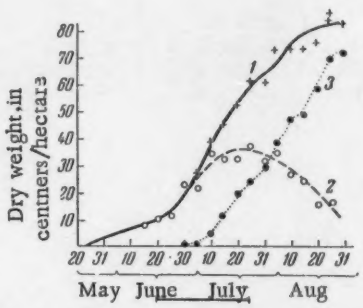

Fig. 6. The course of increase in total dry weight of the harvest (1), dry weight of leaves (2) and tubers (3) in potato plantings. Data obtained by A. G. Lorkh in 1957 and processed by L. E. Strogonov

Using the figures of curve B (Fig. 4) and the total daily amount of radiant energy falling on the plants (Fig. 2), we were able to calculate the daily value for the absolute amount of the energy of photosynthetically active radiation which was absorbed by the plants at various times of the growing period. These figures are given in Fig. 7*; they are represented as points, and are leveled out as the curve R.

Taking the heat of combustion for the dry weight of the harvest as 4000 kcal/kg, we were able to calculate the amount of energy bound in the increased mass of harvest during each five-day interval (figures in row II of Fig. 7). Relating these values to the amount of energy absorbed during each five-day interval, we obtained values (in %) for the efficiency of the absorbed energy used directly in photosynthesis.

These values for a five-day interval are given in Fig. 8 (curve M).

As we can see in Fig. 8, during the period of rapid growth the efficiency coefficient of photosynthesis for photosynthetically active radiation absorbed by the plants reached a high value of 4-5 and even 6%. We should note once more that these high values for the efficiency of absorbed energy corresponded to the period of most intense growth in potato, to favorable weather conditions, and very high increments of growth which finally lead to obtaining a high potato yield. However, the indicated percentages for the use of energy in photosynthesis were all several times lower than that theoretically possible based on a quantum requirement of 8-12 for photosynthesis. These data indicate that even in this particular circumstance when a high yield was obtained we are still inclined to consider that it could be increased.

Curve N, Fig. 8, shows the percentage of the total radiation falling on the plants that was used in photosynthesis (see Fig. 2). As we see, these figures were specially low during the first half of the growing period when the canopy had not yet closed over and much of the energy was transmitted to the soil, and in addition the efficiency coefficient of photosynthesis for the absorbed energy was low.

On the whole, the percentage of the total energy falling on the plants that was used in phtosynthesis was

*The data for the increase in yield were kindly given to us by A. G. Lorkh, and were processed by L. E. Strogonov.

at this time only 0.15-0.17% (curve N) and was 7-8 times smaller than the efficiency coefficient for the absorbed energy (curve M).

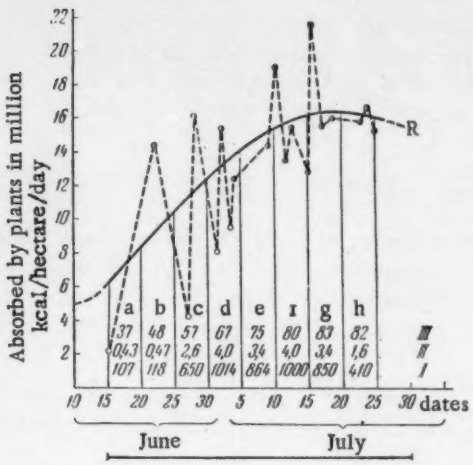

Fig. 7. Absolute amount of photosynthetically active radiation in million kcal/hectare in a day absorbed by potato plants.
Dots — bright days; circles — cloudy days. Figures in rows: I) increase in total dry weight of harvest (kg/hectare) in a five-day interval; II) amount of energy absorbed in million kcal/hectare during the increase in harvest within the five-day interval; III) the amount of energy of photosynthetically active radiation in million kcal/hectare absorbed by the plants during the five-day interval; these were determined graphically on the basis of the area for figures a,b,c,d,e,f,g,h defined by sections of the absissa for each five-day period and the corresponding sections of the curve R.

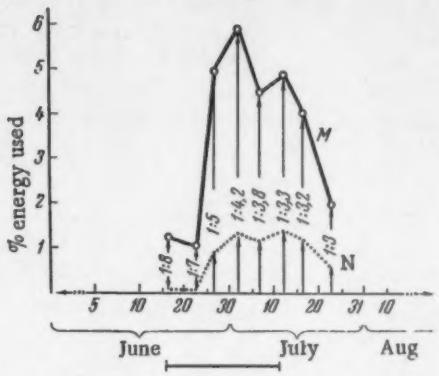

Fig. 8. The percentage of energy of photosynthetically active solar radiation absorbed by the plants which was used in photosynthesis (curve M), and percentage of the total falling on the plants (curve N)
1:8 , 1:7, --------1:3 — the ratio between the value in curves N and M.

It was quite natural that as the leaf area increased and the canopy closed over the difference between these two values became less, and by the 25th of June the ratio between them was 1:3.

Hence, we can try to increase the yield either by increasing the total absorption of energy by the plants during the first half of the growing period, or else by increasing the efficiency coefficient of phtosynthesis. In this particular case any possible progress along the first course would be slight since the leaf area increased almost in accordance with the optimum figures [1].

It would have been possible to get some gain only by hastening the growth of the leaf blades in May and in the first twenty days of June.

As for the second course, i.e. increasing the efficiency coefficient of absorbed energy in phtosynthesis, or to say it differently, increasing the activity of the photosynthetic apparatus, although it would not be easy there would be a greater possibility, as we see it. In this stage investigations of methods, for obtaining high yields in this way should be the primary problem.

The information which we obtained permits us to also come to a conclusion concerning certain additional conditions which are necessary for obtaining high yields. As an example, we might consider water relations as an essential condition. Fig. 7 contains data pertaining to the absorption of photosynthetically active solar radiation by the plants. In reality the leaves also absorb infrared radiation in an amount equal to about 30% of the photosynthetically active radiation. Hence, by increasing the figure for phtosynthetically active radiation absorbed by the plants by 30%, we obtained figures for the absorption of energy of the total radiation. In July these figures varied within the limits of 15 to 30 million kcal/hectare in a day. After subtracting the value for the heat of evaporation of water (586 kcal/kg at 20°) from these we calculated the amount of water which

should be absorbed by the plants to provide for transpiration in order to overcome the risk of leaf overheating, wilting, and retardation of growth.

These figures corresponded to 25-50 tons, or a layer of water 2.5-5.0 mm per hectare per day.

From this it is evident that even at the latitude of Moscow where the amount of energy from solar radiation is not especially high, and is lower that at southern latitudes, plants require a considerable amount of water during transpiration for an optimal development of leaf blades. Actually, Prof. A. G. Lorkh succeeded in obtaining and maintaining a leaf area in potato plantings of 40 and more thousand m^2/hectare by regular watering, in combination with applications of nutrients, primarily nitrogen fertilizer.

SUMMARY

A field photointegrator was constructed in the Photosynthesis Laboratory at the Institute of Plant Physiology, Academy of Sciences USSR; with the aid of this instrument we calculated the amount of radiant energy which was absorbed by potato and corn plantings in relation to the development of leaf area in these plantings. It was shown that different species of plants with a leaf area of almost the same size absorbed more or less the same amount of radiant energy.

When the leaf area had developed to 35-40 thousand m^2/hectare, the percentage of photosynthetically active radiation energy absorbed was 65-75%, and during subsequent increase in leaf area it changed very little.

In a planting of potatoes which had produced a yield of fresh tuber equal to 430 centers per hectare, and the daily increases in dry weight during the period of greatest leaf enlargement had reached 150-200 kg/hectare in a day, 4-6% of the radiant energy absorbed during the day was used in photosynthesis. This was a high coefficient, however it was still considerably lower than that theoretically possible, (based on 8-12 quanta used in photosynthesis) and indicates the great possibilities and perspectives of further investigations concerning the increase of yield.

The values obtained for the energy balance of the plantings by the method described also make it possible to evaluate the water relations necessary for obtaining high yields, and the amount of water required for transpiration by the plants in order to eliminate the possibility of harmful overheating and wilting of the leaves, and the provision of good conditions for photosynthesis and growth.

The authors consider it their imperative but pleasant duty to express sincere appreciation to Prof. A. G. Lorkh for making it possible to carry out the investigation, and for his constant assistance and attention to it.

The authors also express appreciation to L. N. Bella and V. P. Kornil'ev for assistance in making the photointegrator, and to L. E. Strogonov for assistance in obtaining some data concerning the course of yield formation.

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THE ABSORPTION OF CARBON DIOXIDE BY PLANT ROOTS

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As a result of the lengthy investigation of carbon nutrition, it was established by the 'thirties that the basic part in this process is played by leaves and that an insignificant amount of CO_2 may also enter through the root system of plants.

In 1934 Livingston and Beall [13], in summing up their investigations, were the first to calculate the amount of CO_2 entering a plant from the soil. It turned out that roots may absorb five percent of CO_2 or even more, which is fixed in photosynthesis. On the other hand, Overkott [14] finds that the amount of CO_2 obtained through the roots may reach 5 - 20%, and in some cases even 50%.

Towards the end of the 'thirties, the method of isotope labelling became a great help to investigators. Using it, Overstreet, Ruben and Broyer [15] have shown that bicarbonate ions are absorbed by the roots of barley plants. Beginning in 1951, there appeared a series of investigations carried out by A.L. Kursanov and A.M. Kuzin and their collaborators [1 - 8], which were devoted to the absorption of CO_2 by roots and to the further transformations of carbon dioxide in the plant. In these papers, it has also been shown that CO_2 is absorbed by plant roots both from solution and from a gaseous medium. It could be calculated that the amount of CO_2 absorbed by roots may reach $\frac{1}{4}$ of that used in photosynthesis.

The papers of A.L Kursanov and A.M. Kuzin, which again raised the question of the nutrition of plants, emphasizing a peculiar biochemical role of the soil carbon dioxide, and establishing the fact that the amount of CO_2 absorbed through the roots may vary with different conditions of existence, with various species peculiarities and with age of plants, contributed to the further study of this problem.

In 1952, there appeared a paper by Samokhvalov [9], in which the author, using his own extensive experimental material, attempted to prove that "... plants are able to absorb carbon dioxide by the roots directly from the soil, and just as easily and freely as by the leaves from the air". However, the reliability of these laboratory experiments may be somewhat doubtful, because of the insignificant accumulation of dry substance by the plants in the course of the experiments, because of a lack of correlation of amount of CO_2 given to plants with the increase in dry matter, and, finally, because of the fact that, according to the data of the author, the absorption of CO_2 in the dark by plant leaves was equal to, or even higher than, that during photosynthesis. The impression is left that the photosynthesis of the experimental plants was either suppressed by some factor, or was not sufficiently maintained. On the other hand, the field experiments described by Samokhvalov [9] do not solve the problem of the role played by the root system of plants in the assimilation of CO_2.

V.A. Chesnokov and A.M. Stepanova, on the basis of experiments which they carried out, conclude that a certain amount of CO_2 passes into the plant through the root system, but the authors [10] record simultaneously a reverse, and more rapid process of excretion of CO_2 from the root into the soil. The authors also note that the small amount of carbon dioxide which enters the plant through the root cannot provide normal nutrition for the plant.

In a later work of Kursanov and Kulaeva [8], it was noted that the main significance of root fixation of carbon dioxide consists, evidently, in its participation in the di- and tricarboxylic acid cycle, which is at the basis of the absorption activity of the roots.

In the present paper are given some results of work carried out by the author under the direction of O.V. Zalenskii, with the help of I. Mikriukova, a student of Molotovskii State University, on the elucidation of the quantitative interrelationships of CO_2 assimilation by the roots and leaves of plants, and the dependence of these interrelationships on the conditions of illumination. The work was carried out in specially constructed, hermetically sealed chambers made of plastic, and using the radioactive isotope of carbon, C^{14}. Preliminary results of these investigations and a detailed description of the chambers were published in the paper by Zalevskii, Semikhvatora, and Voznesenskii [11].

METHODS

The experiments were carried out with plants of barley, kidney beans and gout weed. The barley and beans were grown on nutrient solution. Three weeks prior to the experiment, the gout weed plants were transferred from the natural environment of germination to the nutrient solution. In various series of experiments, 6 to 15 young barley plants (1-3 leaves, with an area of 5 - 15 cm^2) or 2 plants of beans (11 - 13 days, leaf area 15 - 20 cm^2) or gout weed were placed for the duration of experiment into a closed chamber with a movable β-particle counter. Each plant was placed into an opening in the bottom of the upper chamber, and was sealed in with a soft paste, so that the leaves were in the upper chamber and the roots were in the lower chamber. For complete separation of chambers, a layer of water was placed on the bottom of the upper half; it was established in the course of experiments that a layer of neutral oil was preferable to water. The β-particles counter, established on a movable plane, allowed constant control of the percentage composition of carbon dioxide in the chambers. Carbon dioxide gas, with a specific activity of 0.5 mC/l of CO_2, prepared in a gas tank, was introduced, according to the problem, into either the upper or the lower chamber.

Illumination of plant leaves was carried out with a 3H-500 watt lamp through a water filter, at an intensity of approximately 40 thousand lux. The plant roots were always in a darkened chamber, in which the atmosphere was saturated with water vapor, to prevent drying of roots.

Every series of experiments included four treatments: 1) absorption of carbon dioxide by leaves in the dark; 2) absorption of CO_2 by plant roots, when leaves were darkened; 3) assimilation of CO_2 by illuminated leaves; 4) absorption of CO_2 by plant roots, when leaves were illuminated.

The various series of experiments differed in the duration of the experiment and in the original concentration of CO_2 in the chambers.

After a desirable length of time, the plants were taken out of the chamber, and divided into parts (roots, stem, leaves), which were fixed separately by boiling alcohol.

Calculation of the amount of CO_2 assimilated by the plants was carried out by measurement of the activity of preparations made from powdered plants, and was determined by the β-particle counter according to the decrease of CO_2 concentration in chambers. Corrections to the primary results of the counts/minute from the preparation were made for the background of the counter and for self-abosrption of β-particles in the layer of the prepared substance. Further, by the method we developed [12], starting from the proportion of the total number of counts/minute for the dry plant material and of the CO_2 which has entered into them, the amount of CO_2 which entered into the whole plant and its separate parts during the experiment was calculated.

For elucidation of the question of the effect of leaf illumination on the rate of CO_2 absorption by plant roots from the nutrient solution, to which labelled sodium bicarbonate was added, the barley plants, grown under natural condition, were taken in the tillering stage. For 3 - 4 days prior to the experiment, they were transplanted into a 0.5 Knopp nutrient solution, and were grown in room atmosphere, under the same conditions of artificial illumination which prevailed in the experiment. For one series of experiments, 4 to 6 plants, similar in their development, were selected and two each were used in the "darkened" and "illuminated" treatment of the experiment. For the duration of the experiment the plants were transferred into a sealed chamber, the lower part of which was filled with nutrient solution to which $NaHC^{14}O_3$ was added. The activity of the solution did not exceed 0.3 mC/l. A β-particle counter was attached in the upper part of the chamber, hermetically sealed from the lower one. After the experiment, the roots of the plants were thoroughly washed with water. Then leaves and roots were separately fixed by alcohol, and prepared for the measurement of their radioactivity.

In our first experiments on the balance of carbon dioxide absorbed by plants, we obtained data indicating that roots absorb up to 20 - 30% of the CO_2. But, as later became clear, and as was confirmed by specially prepared experiments, in these experiments there was no complete isolation between the upper and lower chambers. The smallest error in sealing in the plants may basically change the results of the work. Only covering the bottom of the upper chamber with a layer of water or, even better, of oil, made it possible to observe whether bubbling of CO_2 does not take place from the lower chamber into the upper one, and gave a full guarantee that the chambers were hermetically sealed (especially in working with barley plants). For confirmation, experiments were carried out in which CO_2 was introduced only into the lower chamber, while the upper chamber was illuminated and its top was open or closed. In one of the treatments of the experiment, a layer of soil was placed on the bottom of the upper chamber. As a result, it was found that the amount of CO_2, assimilated by plant roots was practically the same, whether the upper chamber was open or closed, when the bottom of the upper chamber was covered with oil, when the upper chamber was closed, and isolation of the bottom was not sufficient, this amount was increased several-fold.

Absorption of Carbon Dioxide from the Air by Leaves and Roots of Plants

All experimental results are summarized in Tables 1, 2, and 3, in which the number of milligrams of CO_2 absorbed by the plant (calculated per plant) under definite conditions of experimental set up is given, as well as the percentage distribution of this amount among the separate parts of the plant.

TABLE 1

Amount of CO_2 (in mg) Absorbed by Kidney Bean Plant and Distribution of Assimilated Carbon in the Plant (in %)

Experimental treatment		Plant parts	2 hours of 0.3% CO_2		2 hours 0.5 % CO_2		1 hour 1.5% CO_2	
conditions of leaf illumination	place of CO_2 introduction		total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %
Darkened	Upper chamber	Leaves	0.016	61	0.057	51	0.032	66
		Stems		39		31		28
		Roots		Traces		18		6
Darkened	Lower chamber	Leaves	0.004	0	0.05	30	0.008	23
		Stems		16		40		23
		Roots		84		30		54
Illuminated	Upper chamber	Leaves	3.4	97	4.2	91	5.7	95
		Stems		3		9		5
		Roots		Traces		Traces		0
Illuminated	Lower chamber	Leaves	0.046	79	0.08	44	0.16	62
		Stems		9		32		25
		Roots		12		24		13

As can be seen from the data given in Table 1, we did not succeed in establishing a relation between the amount of CO_2 absorbed by the plants over the duration of the experiment and the concentration of CO_2 in the chamber atmosphere. Evidently, to complete this work it would be necessary to take more homogeneous plants, and to consider many factors which are involved in the experiment.

For characterizing the results obtained, let us consider the average data, calculated for an experiment one hour's duration (Table 4).

In the "dark" treatments of the experiment, the amount of carbon dioxide which entered the plant was considerably lower than in those cases in which leaves were illuminated (with the exception of gout weed). The distribution of assimilated carbon during the period when leaves are in the dark is determined by the part of the plant exposed to CO_2. The percentage distribution, in relation to the part exposed, decreases either from leaves

TABLE 2

Amount of CO_2 (in mg) Absorbed by Barley Plant and Distribution (in %) of Carbon in the Plant

Experimental treatment			Duration 1 hour						Duration 2 hours				Duration 5.5 hours			
			0,5% CO_2		0,65 CO_2		1 % CO_2		1 % CO_2				1 % CO_2			
Conditioned of leaf, illumination	Place of CO_2 introduction	Plants parts	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %	total CO_2 in mg	distribution in %
Darkened	upper	Leaves	0.0009	90	0.0006	100	0.0008	100	—	—	—	—	0.0038—0.0051	85—89	0.006	88
		Roots		10		0		0						15—11		12
Darkened	lower	Leaves	0.0006	0	0.0006	0	0.0005	0	0.0012	19	0.0014	20	0.007—0.01	38—47	0.008——0.0067	33—45
		Roots		100		100		100		81		80		62—53		67—55
Illuminated	upper	Leaves	0.41	100	0.4	100	0.45	100	0.44	98	—	—	1.2—1.9	90—92	2.0	95
		Roots		0		0		0		2				10— 8		5
Illuminated	lower	Leaves	0.008	88	0.013	90	0.013	96	0.016	80	0.018	80	—	—	—	—
		Roots		12		10		4		20		20				

TABLE 3

Amount of CO_2 (in mg) Absorbed by Gout Weed Plant and Distribution (in %) of Assimilated Carbon in the Plant
Duration of the experiment – 1 hour. Concentration of CO_2 – 0.5%

Experimental treatment		Plant part	Total CO_2, in mg	Distribution, in %
Conditions of leaf illumination	Place of CO_2 introduction			
Darkened	Upper chamber	Leaves	0.026	79
		Stem		6
		Root		15
Darkened	Lower chamber	Leaves	0.026	6
		Stem		15
		Root		79
Illuminated	Upper chamber	Leaves	1.54	93
		Stem		7
		Root		Traces
Illuminated	Lower chamber	Leaves	0.026	14
		Stem		11
		Root		75

to the root, or vice versa. When leaves are illuminated, however, the greatest part of the carbon is contained in the plant leaves, independently of the point of CO_2 introduction.

In gout weed plants, the leaf surface of which was about 1 dm^2, the rate of photosynthesis was very low for some reason. Because of this, when CO_2 was introduced into the lower chamber and leaves were illuminated, there was no stimulation for the introduction of CO_2 through the roots, and its distribution remained the same when the leaves were left in the dark.

TABLE 4

Average Amount of CO_2 (in mg) Absorbed by Plants, Calculated per Hour and Distribution (in %) of Assimilated Carbon in the Plant

Experimental treatment		Plant parts	Kidney beans		Barley		Gout weed	
Conditions of illumination	Place of CO_2 introduction		Total CO_2 (in mg)	Distribution, in %	Total CO_2 (in mg)	Distribution, in %	Total CO_2 (in mg)	Distribution, in %
Darkened	Upper chamber	Leaves		60±5		97±3		79
		Stem	0.02	33±4	0.0008	–	0.026	6
		Root	±0.01	8±7	±0.0001	3±3		15
Darkened	Lower chamber	Leaves		18±12		0		6
		Stem	0.01	25±9	0.0006	–	0.026	15
		Root	±0.01	56±19	±0.00004	100		79
Illuminated	Upper chamber	Leaves		94±2		100		93
		Stem	3.2	6±2	0.42	–	1.54	7
		Root	±2	traces	±0.02	0		traces
Illuminated	Lower chamber	Leaves		62±12		92±3		14
		Stem	0.07	22±9	0.011	–	0.026	11
		Root	±0.05	16±5	±0.002	8±3		75

TABLE 5

Comparative Amount of Carbon Dioxide Absorbed by Plant Leaves and Roots

Experimental treatment		Kidney bean	Barley	Gout weed
Conditions of illumination	Place of CO_2 introduction			
Darkened	Upper chamber	1	1	1
Darkened	Lower chamber	0.5	0.75	1
Illuminated	Upper chamber	160	525	60
Illuminated	Lower chamber	3.5	14	1

In Table 5, it can be seen that the absorption of carbon dioxide by leaves and roots is practically the same in the dark, and makes up from 0.15% to 2% of the photosynthetic rate in the light. When leaves are illuminated, the amount of CO_2 absorbed by plant roots is 2.2% in kidney beans and 2.7% in barley, as compared with the amount of carbon dioxide which is assimilated by leaves in the process of photosynthesis.

It is interesting to look at the data of Table 2 for 1% CO_2 content, in relation to duration of the experiment. For this, we give in Table 6 the calculated average values of distribution of absorbed carbon in leaves and roots of young barley plants. We see that, as the duration of the experiment is increased, a relative accumulation of carbon takes place in those parts of the plant which did not come into direct contact with the CO_2 atmosphere. When carbon dioxide is absorbed in the dark by roots and leaves, an equalization of carbon distribution between them must take place in the plant with time. This process will take place faster as CO_2 is introduced through the roots, as the rate of the ascending flow in the plant (according to the data of Kursanov [5, 6]) is higher than that of the descending flow of plastic substances.

TABLE 6

Percentage Distribution of Assimilated Carbon in the Barley Plant in Relation to Experiment Duration
Original CO_2 concentration 1%

Experimental treatment		Plant parts	Place of CO_2 introduction		
Conditions of illumination	Place of CO_2 introduction		1	2	5.5
Darkened	Upper chamber	Leaves	100	–	87
		Roots	0	–	13
Darkened	Lower chamber	Leaves	0	20	40
		Roots	100	80	60
Illuminated	Upper chamber	Leaves	100	98	93
		Roots	0	2	7
Illuminated	Lower chamber	Leaves	96	84	–
		Roots	4	16	–

Carbon Dioxide Absorption from Aqueous Medium by Plant Roots

The results of three series of experiments in absorption of carbon dioxide by barely plant roots from a nutrient solution, to which $NaHC^{14}O_3$ has been added, are given in Table 7.

The data are given in relative numbers in counts/minute, proportional to the amount of CO_2 absorbed by the plant. We could not recalculate these figures, since we did not know the isotope content of the CO_2 in the nutrient solution.

In the first series of measurements, the specific activity of the solution was about 0.3 mC/l, but it was smaller in the second and third series. The duration of the experiments was 3.5 hours. When the plants were darkened, the appearance of $C^{14}O_2$, and a gradual increase in its concentration with time, was observed in the upper chamber. Since carbon dioxide which is absorbed by roots ascends mainly in the form of organic acids (Kursanov et al. [4]), its release through the leaves in the dark could have been connected with the process of respiration, or simply with partial decarboxylation of organic acids transported from the roots. When leaves are

illuminated, no release of $C^{14}O_2$ into the chamber space was observed and consequently, all carbon dioxide which ascends to the leaves is used by them in the process of photosynthesis.

The amount of carbon assimilated by the plant from the nutrient solution through the roots, during leaf illumination, is approximately three times greater than that during the period when leaves are darkened.

TABLE 7

Amount of Carbon Assimilated by Barley Plants (in counts/minute) From a Nutrient Solution with $NaHC^{14}O_3$ Added and Its Distribution Between Roots and Leaves

Number of the series	Conditions of leaf illumination	Plant parts	Total No. of counts per min. per plant	Distribution in %	Ratio of the amount of CO_2 absorbed to that of darkened plants
1	Darkened	Leaves	3980	31	1
		Roots		69	
	Illuminated	Leaves	11120	63	2.8
		Roots		37	
		Leaves	12720	52	3.2
		Roots		48	
2	Darkened	Leaves	3280	17	1
		Roots		83	
	Illuminated	Leaves	7510	54	2.3
		Roots		46	
3	Darkened	Leaves	845	21	1
		Roots		79	
	Illuminated	Leaves	2310	52	2.7
		Roots		48	

TABLE 8

Average Amount of CO_2 Absorbed by Roots and Leaves of Barley Plant, and Distribution (in %) of Assimilated Carbon in the Plant

Conditions of leaf illumination	Plant parts	Total No. of counts/min. per plant	Corresponding amt. of absorbed CO_2
Darkened	Leaves	23±5	1
	Roots	77±5	
Illuminated	Leaves	55±4	3
	Roots	45±4	

In considering the distribution of CO_2 absorbed by the leaves and roots of the barley plant (Table 8), we saw that in the case of the darkened leaves the main portion of the absorbed CO_2 (77%) was contained in the roots. However, upon illumination of the plants more than 1/2 of the CO_2 was found in the cells.

SUMMARY

The quantitative relations between carbon dioxide absorbed by roots and by leaves was investigated by means of hermetic chambers containing $C^{14}O_2$. The effect of light on this relations was studied. The experiments confirm the findings that roots can absorb gaseous carbon dioxide as well as carbon dioxide from solutions of carbonates. The amount of CO_2 absorbed by roots depends on the type of plant and its state and also on the multifarious factors of the environment of the plant. Illumination of leaves increases the absorption of CO_2 by roots up to 15 times. Nevertheless this amount is always insignificant and our observations show that it is less than 5% of the total amount of carbon dioxide absorbed by the plant.

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TRANSLOCATION OF ASSIMILANTS AND RESPIRATION OF CONDUCTING PATHWAYS IN RELATION TO SOIL MOISTURE

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In an earlier paper [1], it was noted that prolonged soil and atmospheric drought lowers the rate of flow of assimilants from the leaves into the head of summer wheat. These data, obtained under the field conditions of Zavolzh'e, agree with the results of a number of investigations carried out during prolonged drought [2-8]. However, the reverse results were obtained during short-term or shallow drought [9, 10], and some papers proposed a stimulating effect of the water deficit on the flow of sugars and nitrogenous substances [11, 12].

In connection with this problem, and to confirm and make more precise results obtained earlier in 1955, we carried out, under laboratory conditions, a determination of the rate of outflow of assimilants from leaves at different soil moisutre values, for two crops: summer wheat (variety Melianopus 69) and sugar beet (variety Ramonskaia 632). In addition to direct observations of the outflow, we also included in our investigation the determination of the respiration of fibro-vascular bundles, since it is closely connected with the translocation of substances [13, 14]. Here, respiration interested us above all from the point of view of energy metabolism. The available data [15, 16] already indicate a decrease of respiration efficiency under certain conditions of water deficit, or a certain break-down of the linkage of respiration with other physiological processes. However, these data do not touch on the respiration of conducting pathways.

The plants were grown in a vegetative house of Plant Physiology Institute, in Wagner vessels of a capacity of 5.5 kg of absolutely dry soil. Vessels for beet were filled with hothouse soil, for wheat, with peat-podzol soil. A one-tenth part of river sand was added.

In the experiment, there were treatments with optimal conditions of water regime (70% of total moisture capacity of the soil for wheat, and 80% for beet), and with definitely insufficient water supply (25-30% and 35% for wheat, 35% for beet). Up until appearance of the third pair of leaves, the soil in all beet vessels was watered until the moisture level reached 70% of the full moisture-capacity. Then the moisture was gradually brought to 35% for one group of plants, and to 80% for the other. All wheat vessels were at first kept at the moisture level of 60% of the total moisture capacity; after appearance of one or two leaves, the soil moisture treatments mentioned above were started. The plants which received insufficient water supply were therefore exposed to gradual and prolonged soil drought. Insofar as atmospheric relative humidity is concerned, in spite of variations, there was no atmospheric drought. In this respect, the present experiment differed from the one carried out earlier [1] in the field, with summer wheat, in Zavolzh'e, when the plants that were not watered were exposed to the action not only of soil, but of atmospheric drought.

The plants which remained under optimal conditions of water supply received additional fertilization during filling of the vessels, as well as by supplementary feedings throughout the growing period. During filling of the beet vessels, NH_4NO_3, KH_2PO_4, and KCl were introduced, at the rate of 0.22 g N, 0.15 g P_2O_5 and 0.22 g K_2O of active principle per kg of absolutely dry soil; the wheat was given the same salts, at the rate of 0.29 g N, 0.20 g P_2O_5 and 0.29 g K_2O of the active principle.

For observation of translocation of assimilants the method described earlier, using radioactive carbon C^{14}, was used [1]. A number of changes were introduced into that part of the method which concerns exposing leaves

Fig. 1. Plants of sugar beet during period of sugar accumulation. At left) at soil moisture of 80% of total moisture capacity; on the right) at 35%.

to $C^{14}O_2$: $Na_2C^{14}O_3$ was used for obtaining radioactive carbon dioxide; 30% sulfuric acid (0.5 ml for one determination) was used instead of lactic acid. The concentration and amount of the $Na_2C^{14}O_3$ solution were selected in such a way that the gas activity in the wheat chamber would consist of 43 μC/liter, for beet, 19 μC/liter. The total maximal potential concentration of CO_2 in the chamber consisted, as before of about 3% by volume. The chamber for the leaf used in experiments with beet was different from the analogous chamber for wheat. It was about 850 ml in volume, made out of plexiglass, in the shape of a parallelepiped, in accordance with the shape of the beet leaf. The construction of the reagent vessel was not changed in this case.

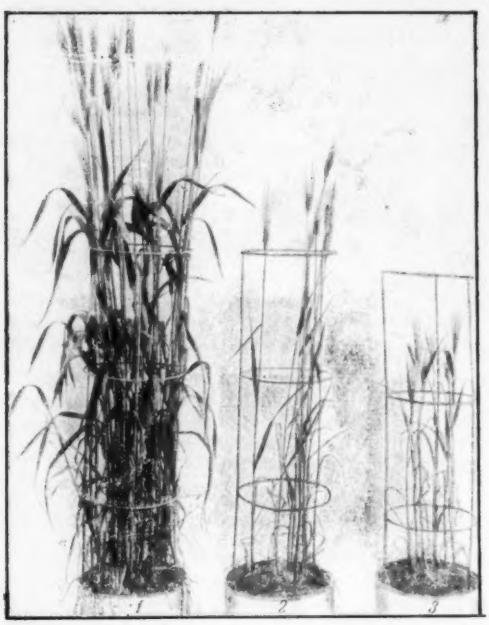

Fig. 2. Plants of summer wheat in the phase of grain formation. 1) At soil moisture of 70% of the total moisture capacity; 2) at 35%; 3) at 25-30%.

The direct measurement of flow was carried out by the method of leaf halves [1]. Flow in sugar beet was determined in the period of sugar accumulation in wheat, during formation and maturation of grain, i.e. in those phases of ontogenesis which are usually characterized by a more rapid translocation of assimilants into the agriculturally valuable organs.

The insufficient water supply greatly retarded the growth of the plants, and somewhat decreased their yield (Figs. 1 and 2, Tables 1 and 2).*

Experimental Results with Sugar Beet

The data of one of the determinations are summarized in Table 3.

The specific radioactivity of a leaf immediately after introduction of $C^{14}O_2$, given in the Table, may characterize its photosynthetic capacity; the activity of leaf tissue one day later indicates the amount of assimilants remaining after a part of them was translocated out of the leaf, or was used up in respiration. The ratio of original specific radioactivity to the activity found a day later gives us an idea of the rate of elimination of photosynthetic products from the leaf. In general, the decrease in assimilants should, in all probability, be taken as due to outflow, since a much greater amount of substances is translocated to other plant organs than is used up in respiration [2].

*The especially great decrease in wheat yield during drought was conditioned by the absence of the tiller shoots, which, under optimal conditions, yield 65% of the total grain collected.

TABLE 1

Yield of Sugar Beet

Soil moisture, in % of total moisture capacity	Weight per root, in g	Amount of sugar per root, in g
80	455	82.4
35	111	22.2

TABLE 2

Yield of Spring Wheat

Soil moisture, in % of total moisture capacity	Weight of grain from 8 plants (1 vessel), in g	Weight of 1000 kernels, in g
70	36.42	41.52
35	5.75	33.40
25-30	1.52	20.50

These data show clearly that the plant leaves are more productive under favorable conditions of water supply than are those under prolonged and intensive drought; in particular, they have a higher assimilating capacity, but also the outflow of photosynthetic products into the root – the organ determining productivity of sugar beet – is considerably increased. Thus, in spite of a small initial productivity, the leaf of a plant which has undergone drought contains, one day later, an even somewhat greater amount of labelled assimilants. One should bear in mind that the expenditure of the plastic substances for respiration was increased in this case, because of the increase in respiration (Table 4 and Fig. 3A). Consequently, the decrease of assimilants due to outflow, in practice, turns out to be even smaller.

TABLE 3

Flow of Assimilants from Leaves and Their Translocation Into the Root in Sugar Beet

Soil moisture, in % of total moisture capacity	Specific radioactivity, in counts/minute per mg dry weight		Ratio (1) : (2)	Radioactivity of the whole root, in counts/minute	Length of petiole, in cm
	Immediately after introduction of $C^{14}O_2$ (1)	One day later (2)			
80	2945	410	4.18	272044	20
35	1704	491	3.47	45102	10

More rapid translocation of assimilants and their more rapid accumulation in the root took place during optimal water supply, even though the pathway from the leaf blade to the root was increased to twice the length (see Table 3, data on the length of petiole). This indicates convincingly that the differences in the flow between the treatments are not conditioned by changes in distance, but by characteristics of the living forces of the plants.

As is known, retardation of outflow of assimilants in turn retards photosynthesis, as a result of the inhibiting action of "overfeeding" [3, 17, 18], and also favors an increase in respiration [19-21]. In our experiment, the leaves of plants exposed to drought showed increased respiration almost throughout the whole period of vegetation (Fig. 3A). Respiration was determined in leaves of one of the middle whorls, according to Warburg, at 30°. In choosing these leaves, we passed consecutively, as the plant was growing, from the lower whorls to the upper ones. Thus, for determinations, we usually used the largest leaves, which had already terminated their growth; analogous leaves received $C^{14}O_2$ when the outflow was studied.

From the curves of Fig. 3, it can be seen that, throughout vegetation, respiration undergoes definite variations. These variations are more or less similar in plants of both treatments. However, the increased respiration, conditioned by the water deficit, is retained.

Differences in the respiration rate of plants of both treatments are clearly seen when respiration is calculated not only per unit weight of leaves, but also per unit nitrogen contained in the tissue, both of total nitrogen

and of protein nitrogen. In Table 4, an example of such a calculation is given for respiration of the fifth whorl of sugar beet in the period of rapid growth of plants.

It is altogether natural that a decrease in assimilation and an increase in respiration have a negative effect on the general balance of organic substance in the plant which experiences moisture deficiency [22].

Thus, in the experiment with sugar beet, we came to the same conclusion as in the experiments carried out earlier with wheat [1, 15, 16].

Simultaneously with observations of translocation of assimilants, we determined the respiration of fibrovascular bundles dissected from leaves. The rapid respiration of conducting pathways is connected with their fulfillment of a basic physiological function – translocation of substances [13 - 14]. However, in our experiments, the fibrovascular bundles had a higher respiration during drought than under optimal conditions of water supply (Figure 3B and C), in spite of the retarded translocation of assimilants which occurred along the bundles. The activity of oxidases, such as polyphenoloxidase, and ascorbinoxidase (Table 5), determined according to the method of Povolotskaia and Sedenko [23], was also increased.

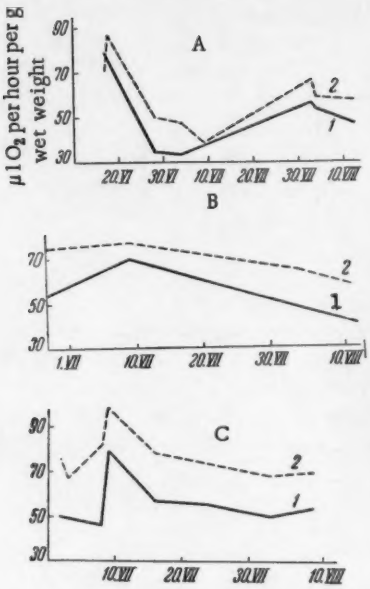

Fig. 3. The respiration rate of leaves (A) and of fibrovascular bundles (B and C) in sugar beet. 1) At soil moisture 80% of total moisture capacity; 2) at 35%.

The significance of fibrovascular bundles for translocation of assimilants is illustrated by microradioautographs (Fig. 4), which were obtained with transverse sections of petioles of the leaves which were exposed to $C^{14}O_2$, and by data on the outflow, which is given in Table 3. Radioactivity in the petiole is concentrated particularly in the vascular bundles, and above all in the phloem. In the photographs shown, it can also be seen that the elements of the phloem are considerably better developed under the optimal conditions of water supply than under drought. A similar increase of development of conducting pathways, and particularly of phloem, during a favorable water regime of plants was noted a number of times [24, 25].

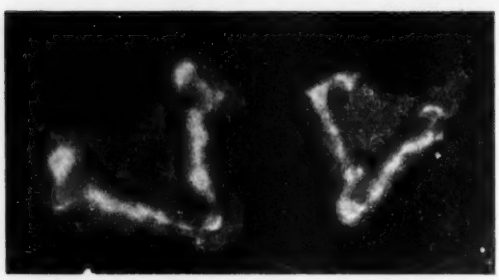

Fig. 4. Microradioautographs of transverse sections of sugar beet petioles, 24 hours after assimilation of $C^{14}O_2$ by leaves. Period of sugar accumulation. At left – soil moisture 80% of full moisture capacity; on the right – 35%.

The conclusion about an increase in respiration of fibrovascular bundles during drought was supported by results which were obtained in an experiment of 1957, set up approximately by the same scheme as in 1955 (Fig. 3B).

Such a lack of correspondence of the level of oxidative processes in conducting pathways and in the rate of translocation of plastic substances along them may mean that, under the unfavorable conditions of drought, a disproportion arises between the separate links of metabolism and affects not only the formation and flow of assimilants, but also the linkage of respiration with other physiological processes. A similar possibility of a decrease in respiration efficiency during drought has been earlier noted by one of us [15, 16].

Experimental Results with Wheat

At a greater soil drought (25-30% of total moisture capacity), the elimination of photosynthetic products and the translocation of assimilants into the spike were retarded throughout the whole period of formation and maturation of grain (Table 6, Figs. 5 and 6).

TABLE 4

Respiration of Sugar Beet Leaves Calculated By Various Methods

Soil moisture, in % of total moisture capacity	Oxygen absorption, in μ liters per hour per g		
	Wet weight of tissue	Total nitrogen	Protein nitrogen
80	34.8	6194.9	6763.9
35	50.3	9747.6	10244.8

TABLE 5

Activity of Oxidative Enzymes in the Fibrovascular Bundles of the Sugar Beet*
(in mg of ascorbic acid oxidized per g tissue in 30 min)

Soil moisture, in % of total moisture capacity	Polyphenoloxidase	Ascorbinoxidase
80	625.0	33.3
35	1632.3	90.0

*Bundles were dissected from the leaves of the sixth whorl in the beginning of sugar accumulation.

TABLE 6

Flow of Assimilants from Leaves and Their Translocation into the Spike in Wheat per Day*

Date of determination, stage of plant development	Soil moisture, in % of total moisture capacity	Rate of elimination of assimilants from leaves**	Specific radioactivity of grain in spike, in counts/minute per mg of dry weight
July 14. Formation of grain ***	70	10.71	252
	25 - 30	4.99	169
July 19. Beginning of grain maturation in the treatment of 25-30%, the end of grain maturation in the treatment of 70%	70	10.19	556
	25 - 30	2.94	217

*$C^{14}O_2$ was assimilated by a leaf of the eighth whorl.
**In this column is given the ratio of original specific radioactivity of a leaf to that found a day later.
***In distinguising the phases of "formation" and "maturation" of grain, we followed the classification of Kuleshov [26], in which the period of grain maturation extends only to the milk stage.

For example, in the radioautograph shown (Fig. 5), it can be seen that during favorable water conditions the spike shows noticeable radioactivity 6 hours after assimilation of $C^{14}O_2$ by the upper leaf, while in the plant grown under drought, the spike was not even defined, and all radioactivity stayed in the assimilating leaf. Meanwhile, the spike was actually almost entirely in the axil of the leaf receiving $C^{14}O_2$.

However, during less intense drought (soil moisture 35% of total moisture capacity) during the period of grain formation, a rather considerable increase in the rate of translocation of assimilants into the spike was noted. Later, during grain maturation, the increase gives way to a decrease, and the plants of this treatment behave like the plants which have experienced a more intense soil drought, i.e. when the soil moisture was 25-30% of total moisture capacity (Fig. 7).

In spite of the possibility of similar periods of intensified translocation of assimilants into the spike, the yield and absolute weight of grain were decreased (Table 2). This is connected with a very considerable decrease in assimilating surface, as well as with retardation of outflow during the phase of grain maturation.

The nature of translocation of assimilants in wheat, at various soil moisture values, shows clearly the possibility that almost reversible physiological changes may arise on insufficient water supply [16].

The distance from the leaf receiving $C^{14}O_2$ to the spike was, on the average, the following: in plants with 70% soil moisture – 27.5 cm, 35% – 24.5 cm, and 25-30% – 7.0 cm. Therefore, as in sugar beet, changes in the

Fig. 5. Radioautograph of the upper part of summer wheat plants showing translocation of assimilants into a spike 6 hours after assimilation of $C^{14}O_2$ by upper leaf. The phase is the beginning of grain maturation. At left – with soil moisture 70% of total moisture capacity; on the right – at 25-30%.

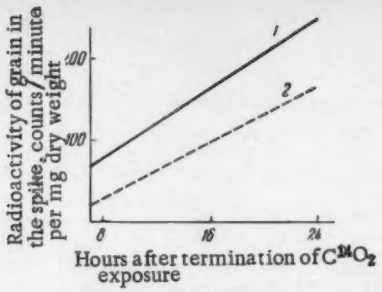

Fig. 6. Translocation of labelled assimilants into the spike of summer wheat. The phase of grain formation. The leaf of the eighth whorl (the uppermost) assimilated $C^{14}O_2$. 1) With soil moisture 70% of total moisture capacity; 2) at 25-30%.

rate of translocation of photosynthetic products cannot be determined only by the length of the pathway from the assimilating leaf to the organ which is accumulating the assimilants. The main role in these changes is evidently played by metabolic factors.

SUMMARY

Investigation of the translocation of the products of photosynthesis carried out with C^{14} shows that optimal water conditions favor, whereas prolonged and intense soil moisture deficiency, retards the flow of assimilates from the leaves to the roots in sugar beets or to the ear in wheat.

Under conditions of optimal water supply the crop yield is larger not only as a result of enhanced leaf growth but also as a result of higher productivity per unit leaf area since in this case the photosynthetic rate and the flow of the products from the leaves is much higher.

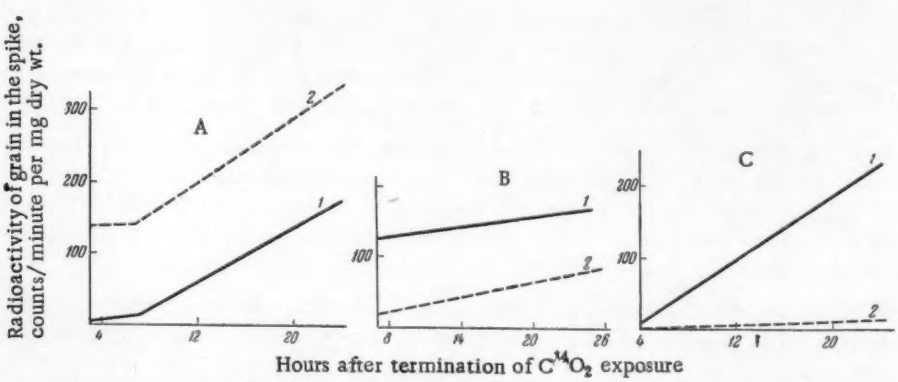

Fig. 7. Translocation of labelled assimilants into the spike of summer wheat. $C^{14}O_2$ was assimilated by the leaf of the eight whorl (the uppermost). A) Phase of grain formation July 13; B) grain maturation, July 22; C) grain maturation, July 27; 1) soil moisture 70% of total moisture capacity; 2) soil moisture 35%.

Retainment of the assimilates in the leaves of plants suffering from drought slows down in turn photosynthesis and leads to an increase of respiration. Consequently in general balance of organic substances is adversely affected. The ultimate result of all these factors is a decrease of the crop yield.

The data obtained in the present study indicate the possibility of accelerating the movement of assimilates under not very stringent drought conditions. Thus these data once more show how manifold and seemingly contradictory are the physiological changes which may be caused by water difficiency under various conditions.

Enhanced respiration was observed in the fibro vascular bundles of sugar beets despite the fact that movement of assimilates was smaller. If one assumes that in normal conductive tissues translocation of substances is related to respiration, one is forced to conclude that in the given case enhanced respiration is to a cergain extent physiologically useless.

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SEASONAL DYNAMICS OF ASCORBIC ACID IN LEAVES OF PLANTS UNDER POLAR CONDITIONS

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The ascorbic acid content of the same plants grown in the same place undergoes seasonal and daily changes, depending on metereological conditions and on the effect of phases of plant development.

The study of the dynamics of ascorbic acid in the plant throughout the vegetative period under polar conditions is important for practical considerations. Up to the present time, hypovitaminosis still occurs in the North, and because of that, it is important to know the optimal periods for using plants in terms of their nutritional vitamin value.

For enrichment of food with ascorbic acid in the North, the rhubarb, onion, and sorrel may be used. Leaves of these plants may be used from the very beginning of sprouting.

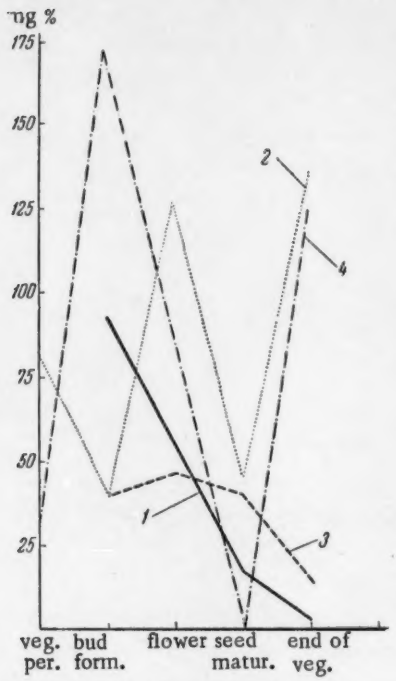
The determination of ascorbic acid in forage plants is important for the cattle industry, in spite of the fact that animals are capable of the biosynthesis of ascorbic acid. The pasture period in the North is very short. In view of the 8-9 monthes in which animals must be maintained in stables, there is great value in the rapid enrichment of animal organisms with ascorbic acid during the summer period, and in obtaining a high vitamin content in silage and other types of feed during the winter period. This has a favorable effect on the productivity, reproductive capacity, and wintering of cattle [1].

The study of the dynamics of ascorbic acid in leaves has also a theoretical significance, indicating the physiological role of ascorbic acid.

There are not many data on the ascorbic acid content of plants grown beyond the Arctic Circle. The data in the literature on seasonal dynamics of ascorbic acid are controversial, and up till now there is no single opinion concerning the effect of phases of development on the ascorbic acid content. Some investigators [2] consider that the changes in ascorbic acid content during the vegetative period take place differently in different groups of plants: in some plants the maximal amount of ascorbic acid is gradually increased towards the end of vegetation, in others, a decreased content of ascorbic acid is observed during flowering, followed by an increase after flowering. In grape leaves, Siniakova [3] observed a maximal amount of ascorbic acid in the end of vegetation, at the time of physiological maturity of the fruit. Most investigators [4 - 14] observed an increase in ascorbic acid content with growth and development of plants, with a maximum towards the period of flowering and a decrease towards the end of the vegetative period. We studied the seasonal dynamics of ascorbic acid in perennial herbaceous plants having value as foodstuff (onion, rhubarb, sorrel, lungwort), as forage (Heracleum, Alopecurus, Roegneria, Rhaponticum), and high in vitamin value (primrose, Dodecatheon). The work was carried out during the growing season of 1955, in the Arctic-Alpine Botanical Garden.

We made determinations of the seasonal dynamics of ascorbic acid in 18 plants. In the large-leaved Heracleum, the leaf mash from the middle part of the leaf blades was analyzed, in grasses – the whole aerial part, and in other plants – the leaf blades. The samples of plants were taken at 8 a.m. Ascorbic acid was determined in the freshly collected samples according to the method of Til'mans, with correction for reducing substances.

The data obtained are given in the Table and in the Figure.



The effect of phases of development on ascorbic acid content in plant leaves. 1) Lungwort; 2) Heracleum pastinacifolium; 3) Alopecurus; 4) rhubarb.

As can be seen by the data given in the Table and in the Figure, the plants investigated may be divided into two groups. The first includes plants with maximal ascorbic acid content in the beginning of vegetation: Heracleum Lehmannianum, Heracleum dissectum, Allium altaicum, Allium Ledebourianum, Allium Schoenoprasum, Allium victorialis, Sanguisorba alpina. The second group includes plants in which maximal accumulation of ascorbic acid was observed not from the very beginning of vegetation, but in a later period: Polygonum Weyrichii, rhubarb, sorrel, Alopecurus, Roegneria, Heracleum pastinacifolium, Allium obliquum, Dodecatheon, Primula, Rhaponticum.

From the list of plants in these groups, it can be seen that there is no strict connection between the nature of ascorbic acid accumulation and any particular family to which the plant may belong. Plants of the same family may fall in different groups.

Throughout the vegetative period the ascorbic acid content in leaves undergoes considerable variations, most noticeable in the plants of high vitamin value: Dodecatheon (varying by a factor of eleven) and Primula (by a factor of six).

The general direction of change in ascorbic acid content is a decrease towards the end of vegetation. However, in the majority of plants in September, after the onset of considerable frosts, an increase in ascorbic acid was observed, which, however, did not reach the maximal value. What determines such an increase in ascorbic acid content in leaves at the end of vegetation, has not yet been established. It is possible that it depends on several factors, one of which is the reaction of the plant to low temperature. The phenomenon of ascorbic acid increase in plants in the end of vegetation was noted in the literature [8, 9, 15]. For the woody undergrowth plants, Kuramshina [15] explains the shift by the fact that after frosts, the sampling is done with the leaves of younger shoots. One may agree with this explanation on the effect of age, since in many of our herbaceous perennials the older leaves die after frost, as a result of which samples are selected from younger leaves, containing more physiologically active substances. The increase in ascorbic acid in the fall is explained by Kuznetsova-Zarudnaia [8] by a renewal of photosynthesis in connection with the onset of clear sunny weather in September.

We have observed considerable variations in ascorbic acid throughout the period of vegetation, but the variations were within certain limits, characteristic for the plants of each family. Thus, for example, in lungwort, plants of low vitamin content Borage family, the ascorbic acid content varies within the limits of 1.4 - 82 mg%; in Rhaponticum, from the low-vitamin Composite family, variations from 7 to 36 mg% were noted, in Sanguisorba, from the higher vitamin-content Rose family – from 13 to 252 mg%, and in Primula and Dodecatheon, plants from the high-vitamin Primula family within 175 - 1002 and 126 - 1417 mg%, respectively.

Thus, in plants of the high vitamin content families, even in the phase of minimal vitamin content, the amount of ascorbic acid is greater than in the plants from low-vitamin families in the phase of maximal content.

For practical considerations, it is important to note that in the forage plants investigated (sorrel, rhubarb, onions, lungwort), the maximal amount of ascorbic acid occurs in the young leaves in the first half of the vegetative period. The comparative stability in ascorbic acid content of the onions is also important. In the five species of onion investigated the variations were the following: Allium altaicum 32 - 82, A. Ledebourianum 28 - 88, A. Schoenoprasum 33 - 106, A. victorialis 33 - 102, and A. obliquum 40 - 109 mg%. These comparisons were made by calculating on a fresh weight basis. However, when calculated on an absolutely dry weight basis, all ratios stay almost the same, except for earliest and latest samples, where, as a result of large variation in the water content, the figures of absolutely dry weight give values relatively higher for the first spring samples (Allium

TABLE

Effect of the Phases of Development on the Ascorbic Acid Content in Plant Leaves
(in mg% of fresh and absolutely dry weight)

Family and name of plants	Fresh wt. according to the phases of development					Absolutely dry wt. according to the phases of development				
	veg. per.	bud. form.	flower	matur.	end of veg. per.	veg. per.	bud. form.	flower	matur.	end of veg. per.
Borage family (Boraginaceae)										
Pulmonaria mollissima Kerner	—	93	54	17	2	—	626	340	111	13
Buckwheat family (Polygonaceae)										
Polygonum Weyrichii F. Schmidt	61	82	33	91	79	529	688	461	570	599
Rheum officinale Baill	30	172	89	0	124	257	1254	761	0	650
Rumex arifolius All.	62	90	50	21	41	687	838	456	195	359
Grass family (Gramineae)										
Alopecurus Seravschanicus Ovcz	—	41	46	40	15	—	210	195	145	54
Roegneria angustiglumis Nevski	57	100	—	169	44	366	479	—	460	125
Carrot family (Umbelliferae)										
Heraclium Lahmannianum Bge.	129	—	92	2	79	1002	—	773	12	301
Heracleum pastinacifolium C. Koch	83	40	127	44	130	716	483	825	324	586
Heracleum dissectum Ldb.	137	112	65	40	39	1157	877	382	231	257
Lily family (Liliaceae)										
Allium altaicaum Pall.	32	48	43	54	83	801	672	395	556	499
Allium Ledebourianum Roem et Schult.	63	42	43	27	66	626	330	395	186	475
Allium Schoenoprasum L.	78	82	35	46	33	914	777	274	488	237
Allium victorialis L.	95	102	78	32	56	956	866	472	222	380
Allium obliquum L.	59	99	94	41	109	681	753	624	342	805
Primorose family (Primulaceae)										
Dodecatheon Meadia L.	774	1198	1417	415	722	6546	10229	13194	3131	6227
Primula elatior L.	334	—	540	1002	833	2732	—	5167	6158	4244
Rose family										
Samguisorba alpina Bge.	253	—	—	13	118	2379	—	—	61	580
Thistle family (Compositae)										
Rhaponticum carthamoides Willd Iljin	—	34	22	36	20	—	216	150	238	126

altaicum, A. Schoenoprasum, A. victorialis, A. obliquum, rhubarb, Alopecurus) and for the autumn samples, somewhat lowered values (Alopecurus, Roegneria, Polygonum, rhubarb, Heracleum Lehmannianum).

SUMMARY

The content of ascorbic acid in leaves of plants is not constant throughout the vegetative period. Seasonal variations of ascorbic acid depend on the plant. Some plants contain maximal amounts of ascorbic acid at the beginning of the vegetative period, whereas in other plants the maximal content of ascorbic acid is observed at later stages. In such edible plants as rhubarb, sorrel, lungwort, onions (but not garlic the maximal content of ascorbic acid is observed in spring at the period when they are used.

In all plants the amount of ascorbic acid in leaves diminishes towards the end of vegetation; in many cases, however, there is an increase of ascorbic acid in September when prolonged autumn frosts set in (Polygonum, Rheum L., Rumex L., Heracleum L., Allium Ledebourianum, Allium victorialis, Allium sativum, Dodecatheon., Sanguisorba L.).

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BRIEF COMMUNICATIONS

DYNAMICS OF CARBOHYDRATES IN LEAVES OF DVURUCHKI*-WHEATS IN THE COURSE OF DIRECTED CULTIVATION

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Up to the present time experimental work on the transformation of the nature of plants has, in general, dealt with transformation of the spring forms of cereals into winter forms or, vice-versa, of winter into spring forms.

With respect to this problem, there has been a complete lack of investigation of a group of plants, so interesting from the biological point of view, such as the barleys and dvuruchki-wheats, which are able to winter out when planted in the fall, and to head when planted in the spring. The adaptation of dvuruchki to growth in regions with light winters caused some investigators [1, 2] to suggest that dvuruchki represent spring forms with a somewhat increased frost-resistance.

However, the ability of some of the dvuruchki to winter out even in a snowless winter, when the air temperature falls to -30°, and to head even after late spring plantings led Liashchenko [3] to propose that dvuruchki may represent a special group of plants, with a widened range of reaction to the greatly varying conditions of the external medium. Undoubtedly this property had arisen in the course of historical development, and in a number of cases is relatively conservative.

In nature, however, forms of dvuruchki with plastic heredity often occur. They represent a valuable starting material for the creation of new, more winter-resistant forms of cereals.

The dvuruchki-wheats which I.F. Liashchenko isolated from the winter variety of Kirgiz State Selection Experimental Station, Eritrospermum 3 (line 7-3), Eritrospermum 9 (lines 25-3 and 25-4) and from a winter wheat of Armenia, variety Amadanikum (lines 106-26 and 97-6) were cultivated for 10 years both as spring and as winter wheats. After ten years of such directed cultivation, seed of all experimental lines from both spring and winter plantings were planted simultaneously in the same background – the autumn and the spring. As a control, the original variety Eritrospermum 3 and 9, Amadanikum and a region-adapted variety of spring wheat, Al'bidum 43, belonging to the same strain as the original varieties of T. vulgare, were planted. After spring planting, lines 7-3, 25-3 and 25-4, of the winter cultivation, as well as the original varieties, remained in vegetative state. On the other hand, the plants from spring cultivation, and lines 97-6 and 106-26 of both spring and winter cultivation, headed simultaneously, and terminated their life cycle 5-10 days later than the region-adapted variety of the spring wheat Al'bidum 43.

After the fall planting of lines 7-3, 25-3, and 25-4, of the spring cultivation, 90-100% were killed by frost in the course of winter period; 50-70% of lines 97-6 and 106-26 and 100% of spring wheat Al'bidum 43 were killed.

Plants of all lines of wheats of winter cultivation have endured the winter normally, and terminated their life cycle 3-5 days earlier than the region-adapted variety of winter wheat, Odesskaia 3.

*Transliteration of Russian – Translator's note.

It follows from the facts stated above that, depending upon the conditions of cultivation and selection, plants of lines 7-3, 25-3, and 25-4 became either winter or spring varieties. At the same time, the ten-year directed cultivation of lines 97-6 and 106-26 did not lead to any substantial change in their nature, which indicates the great conservativeness of their heredity.

In the present work are given the data from the investigation of the dynamics of the qualitative composition and quantative content of individual sugars in winter wheats Eritrospermum 3 and 9, Amadanikum spring wheat Al'bidum 43, lines of dvuruchki-wheats 7-3, 25-3, and 25-4, which lean either towards winter or spring habit, and the typical dvuruchki lines 97-6 and 106-26. In addition, the composition of sugars was determined in leaves of dvuruchki-barley Pallidum 13022, of winter and spring cultivation, and in a typically spring barley, Donetskii 650. Determinations of sugars in plant leaves were made during the fall period from 1955 to 1958.

The qualitative sugar composition was established by means of chromatographic separation on paper. As solvent, we used a mixture of n-butanol, acetic acid, and water (4:1:5). As developers, we used phthalic aniline for reducing sugars, and resorcinol-aniline for nonreducing sugars. The sugars were determined quantitatively by the method of Hagedorn-Jensen; sucrose content was determined after 6-minute hydrolysis with 2% HCl at 70° C. We judged the relative amounts of fructose and raffinose by the intensity of coloration and size of the chromatographic spots.

Results of analyses have shown that the following sugars are found in the leaves of winter wheats Eritrospermum 3 and 9, and Amadanikum, and spring wheat Al'bidum 43: glucose, fructose, sucrose, and raffinose. As stable cold weather is established, at the end of November, the trisaccaride raffinose sharply increases in winter wheats and completely disappears in spring wheat.

We also noted a complete absence of raffinose in the autumn period in the spring barley Donetskii 650, and considerable, progressively increasing, amounts of it in the dvuruchki-barley Pallidum 13022 of winter and spring cultivation. Our data on sugar composition in winter varieties of wheat under low temperature conditions agree with the data of Gunar and Sileva [4]. However, the presence of raffinose in the leaves of spring wheats derived from dvuruchki did not contribute to an increase in their frost resistance. With the onset of stable cold, they perish to a considerable degree, even when they contain a larger amount of raffinose.

This does not support the position [4] concerning a special role of raffinose in the process of hardening and wintering of winter cereals.

It also seemed interesting to follow the dynamics of sugar content in the leaves of the wheat varieties we studied.

Towards the time of onset of stable cold, the winter forms of cereals, in contrast to spring forms, accumulate considerable amounts of soluble carbohydrates [5-7]. It is considered that the latter play a role in the process of wintering.

Considering the nature of the dynamics of total soluble sugars, given in Figure 1A and B, it can be seen that the winter wheats Eritrospermum 9 and Amandanikum differ very considerably from the spring wheat Al'bidum 43 in accumulation of water-soluble carbohydrates. Throughout the whole period of investigation, the winter varieties contained a larger amount of soluble carbohydrates than did spring varieties, in which a greater expenditure of carbohydrates for growth and energetic processes was observed.

As a result of cultivation on the background of winter habit, lines 7-3 and 25-4 inclined towards the behavior of their original varieties in their quantitative sugar content.* After November 20, with the onset of low temperatures of from -5° to -10°, the amount of carbohydrate continued to increase, while a certain decrease in carbohydrates was observed in the original variety. By December 18, line 7-3 contained 5.5% more carbohydrates, and line 25-4 – 3.2% more carbohydrates, than did Eritrospermum 9. Both lines exceed the original varieties in winter resistance, and line 7-3 is more winter-resistant than line 25-4; correspondingly, the plants of line 7-3 accumulate larger amounts of carbohydrates.

*In physiological properties and carbohydrate dynamics, line 25-3 is analogous to line 25-4, winter variety Eritrospermum 3 is similar to winter variety Eritrospermum 9, and line 97-6, to line 106-26. Because of this, we give data only for line 25-4, winter variety Eritrospermum 9, and line 106-26.

The 7-3 of spring cultivation, having become a typically spring form, is similar to the spring variety Al'bidum 43 as regards carbohydrate dynamics; line 25-4 of spring cultivation, as regards carbohydrate dynamics, is analogous to the winter variety Eritrospermum 9, while in quantitative content it occupies an intermediate position between Eritrospermum 9 and Al'bidum 43, showing a certain tendency to lean towards the habit of the spring variety.

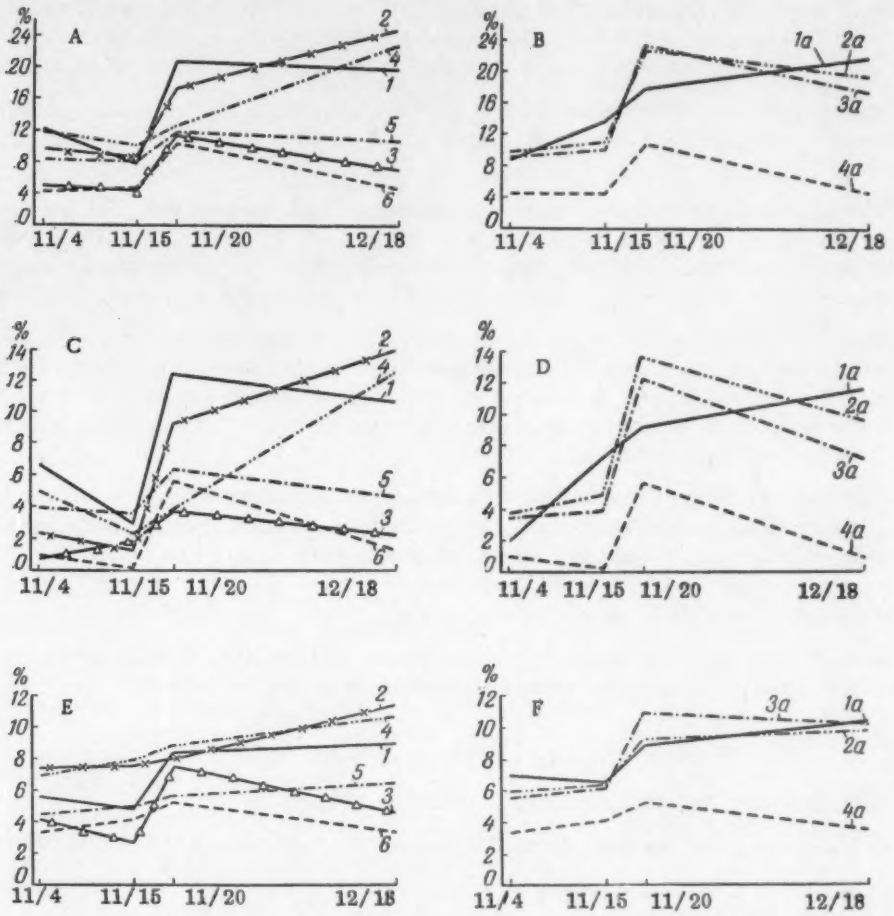

Fig. 1. Dynamics of total soluble sugars (A and B), sucrose content (C and D), and glucose content (E and F) in leaves of winter and spring wheats obtained from dvuruchki (A, C, and E) and of dvuruchki wheat (B, D, and F), planted in the fall, in % of absolutely dry weight. 1) Eritrospermum 9; 2) line 7-3 of winter cultivation; 3) line 7-3 of spring cultivation; 4) line 25-4 of winter cultivation; 5) line 25-4 of spring cultivation; 6) Al'bidum 43; 1a) Amadanikum; 2a) line 106-26 of winter cultivation; 3a) line 106-26 of spring cultivation; 4a) Al'bidum 43.

The similarity of the qualitative composition of sugars in lines 7-3 and 25-4 of spring cultivation to that of the original varieties, and the similarity of the dynamics of carbohydrate accumulation in line 25-4 to that of the original varieties, indicates that, even though they have become typically spring varieties, the plants of these lines have not completely lost the properties of carbohydrate metabolism characteristic of the winter varieties. This, evidently, reveals one aspect of the conservatism of carbohydrate metabolism.

The cultivation of dvuruchki wheats 97-6 and 106-26 on winter and summer background did not bring about a change in their nature; they remain typically dvuruchki even after ten years of directed cultivation. Having

a similar qualitative sugar composition, the dvuruchki wheats differ only insignificantly among themselves in quantitative content of soluble carbohydrates. They accumulate 2.4-4.5% less carbohydrates than the winter variety Amandanikum, and are less winter-resistant than that variety.

The dynamics of sucrose accumulation in lines 7-3, 25-4 and 106-26 (see Fig. 1C and D) are analogous to the dynamics of accumulation of the total sum of sugars.

From the data of Fig. 1E, it follows that, insofar as the dynamics of glucose accumulation is concerned, the winter lines considerably exceed the winter variety Eritrospermum 9 throughout the whole period of the investigation. Line 25-4 of spring cultivation is analogous to Eritrospermum 9 with respect to the nature of the dynamics of glucose accumulation, while line 7-3 is analogous to the spring variety Al'bidum 43.

With respect to the quantitative content of glucose, towards December 18, they occupy an intermediate position between the spring and the winter variety, and in line 7-3 a tendency towards the spring variety habit is clearly seen.

In the typical dvuruchki wheats (see Fig. 1F), the greatest glucose content is noted in the spring line. The winter line and the winter variety Amandanikum contain approximately the same amount of glucose. In contrast to the spring line, the glucose content in the winter line and in the winter variety Amandanikum continues to increase with the onset of cold weather, while in the spring line, the glucose content is gradually diminished.

Thus, from the data given above, it can be seen that, in the dvuruchki wheats cultivated in the winter habit, the nature of the dynamics of carbohydrate metabolism retains the characteristics peculiar to the original forms of winter wheats. Quantitatively, the content of the total sum of carbohydrates and of their individual forms is somewhat higher in the dvuruchki wheats than in Eritrospermum 9, which, along with other factors, brings about their increased frost resistance.

Dvuruchki wheats of spring cultivation, having developed towards the habit of spring varieties, retain to a certain degree the characteristics of carbohydrate metabolism which are peculiar to the winter forms. The qualitative composition of sugars in dvuruchki wheat lines 97-6 and 106-26, and line 7-3, 25-3 and 25-4 of winter and of spring cultivation differs from the qualitative composition of sugars in the spring wheat Al'bidum 43, and is analogous to that of winter wheats.

The nature of carbohydrate metabolism in the transformed and in the typical dvuruchki wheats suggests that it is incorrect to consider dvuruchki as spring forms with increased frost-resistance.

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DEPENDENCE OF RESISTANCE OF PLANTS TO HIGH AND LOW TEMPERATURES ON THE QUALITY OF NITROGEN NUTRITION

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In a study carried out together with Genkel' [1], we have established that anions and cations have different effects on the colloidal-chemical properties of protoplasm and on the resistance of plants to unfavorable conditions of the external medium. The observed facts caused us to suggest a specific action of cations and anions which conditions the structural or hydrophylic viscosity of the protoplasm in plants.

In the light of the facts established, we have carried out investigations of the effect of various forms of nitrogen nutrition on the colloidal-chemical properties of the cells of the tobacco plant.

The well-known investigations of Prianishnikov [2] have established a dissimilar physiological significance of ammoniacal and nitrate forms of nitrogen in the mineral nutrition of plants. The subsequent investigations of Vladimirov [3] have shown that metabolism, in such plants as tobacco, changes sharply in accordance with the form of nitrogen. According to the data of this author, the nitrate form of nitrogen, as compared with ammoniacal, increases the content of sugars, proteins, and organic acids in tobacco leaves, while the amount of nicotine in them is decreased.

In our investigation, we decided to follow the changes in the colloidal-chemical state of the cell protoplasm, and to determine the degree of resistance of tobacco plants to high and low temperatures, as they are given different forms of nitrogen nutrition.

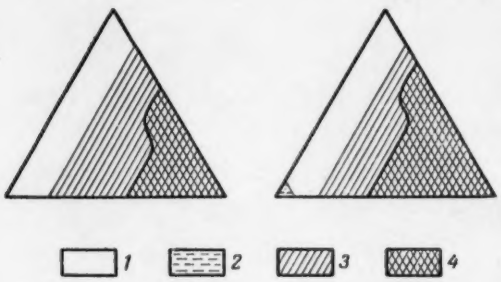
The experiment was carried out with tobacco, (Nicotiana rustica), variety Ermakovka. The complete dose of fertilizers, NPK, was applied to the soil of the vessels prior to planting. In one treatment, a nitrate form of nitrogen, $Ca(No_3)_2$, was used, in the other an equivalent amount of $(NH_4)_2SO_4$. The soil moisture was held at the level of 60% of the total water capacity.

The viscosity and elasticity of protoplasm, the degree of hydrophylic property of colloids, and the amount of free and bound water were used as indicators of changes in the colloidal-chemical properties of cells. Along with this, the degree of resistance of plants to high and low temperatures was determined.

The protoplasmic viscosity was determined by a plasmolytic method, according to the time of transformation of concave plasmolysis into convex [4], while protoplasmic elasticity was determined by the method of centrifugation, proposed by Genkel' [5]. The hydrophylic property of colloids was determined according to the method of triangular diagrams of Dumanskii [6], the total organic acids, by the method of Shmuk [7], heat resistance of plants, by the method of Genkel' [8].

The frost resistance of plants was determined by a method we developed, the technique of which can be described as follows: several test-tubes filled to $\frac{1}{3}$ with distilled water were taken for the experiment. Ten to twenty sections of the upper (or lower) epidermis of the leaf were immersed in the test-tubes. After that, all test-tubes were placed into a cryohydric solution (7 g K_2SO_4 per 100 g water), with a temperature of -1.55°, which was kept in the refrigerator or in a cooling mixture of ice and rock salt. In order to prevent too much cooling, small pieces of ice were placed in each test-tube, two or three minutes after placing them into the cryohydric solution. After a certain period of time (in our experiments in 5 minutes), two test-tubes were

transferred from the cryohydric solution into a beaker filled with water at 18-22°, for thawing. After careful thawing, the sections were placed in a solution of neutral red (1 : 5000) for 10 minutes. Then the sections were transferred into a molar sucrose solution, and were examined under a microscope. The absence of plasmolysis in the cells indicated their death in the definite amount of time at the temperature mentioned above. The leaves of the fifth to seventh whorl from below were used for the experiment. At this period, the experimental plants were in the phase of flower-bud formation.



Triangular diagram of coagulation of hydrosol of tobacco leaves, variety Ermakovka. At the left) the plants given nitrate nitrogen; on the right) plants given ammoniacal nitrogen: 1) separation into layers; 2) clouding; 3) incomplete coagulation; 4) complete coagulation.

Before approaching directly the exposition of the experimental data obtained, we should mention that the plants grown on the nitrate form of nitrogen were characterized by more rapid growth and by greater size of leaf blades, and bloomed 5-7 days earlier than plants grown on the ammoniacal form of nitrogen.

The results of determination of the hydrophylic properties of colloids are given in the figure. As the data of the figure show, the area of complete coagulation of colloids, which characterizes the degree of colloidal hydrophylly is greater in the plants given the ammoniacal form of nitrogen than in the plants given the nitrate form of nitrogen. Thus, on the basis of these data, we may conclude that the degree of hydration of cell colloids changes in accordance with the quality of nitrogen nutrition of plants. In plants grown on the ammoniacal form of nitrogen, the hydrophylic property of colloids is higher than in those plants which were grown on the nitrate form of nitrogen. The changes of cell hydration during growth of plants under different conditions of nitrogen nutrition are reflected in the viscosity and elasticity of plant cells, which can be seen from the data of Table 1.

TABLE 1

The Effect of the Quality of Nitrogen Nutrition on the Content of Organic Acids, Viscosity, Elasticity, and Resistance to High and Low Temperatures of Cell Protoplasm in Tobacco Leaves
(average of five determinations)

Source of nitrogen nutrition	Total organic acids, in g malic acid per 100 g dry weight	Viscosity of protoplasm (time of transformation of plasmolysis, in minutes)	Elasticity of protoplasm (centrifugation time necessary for protoplasm separation, in minutes)	Heat resistance (temperature at which cells die)	Cold resistance (time for the death of cells at -1.55°, in minutes)
$Ca(NO_3)_2$	6.60	52	5	41	25
$(NH_4)_2SO_4$	1.02	83	10	43	20

As the data of Table 1 show, the viscosity and elasticity of protoplasm increases noticeably in plants grown on the ammoniacal form of nitrogen. Consequently, different forms of nitrogen have a great effect on such important colloidal-chemical properties of the cell as viscosity and elasticity of protoplasm. It is important to note that, in plants given the ammoniacal form of nitrogen, a simultaneous increase in the degree of hydration of colloids is observed, as well as in protoplasmic viscosity. In the same table are given the data from the determination of resistance to high and low temperatures of tobacco leaves grown under different conditions of nitrogen nutrition.

It can be seen from Table 1 that the degree of resistance of plants to high and low temperatures changes in accordance with the quality of nitrogen nutrition. Thus, for example, the heat resistance of plants is noticeably increased on ammoniacal nutrition, and simultaneously cold resistance is decreased.

The increased heat resistance and decreased cold resistance in plants given ammoniacal nitrogen is accompanied by an increase in protoplasmic viscosity, and, vice-versa, the decreased heat resistance and increased cold resistance in plants given nitrate nitrogen is accompanied by a decrease in protoplasmic viscosity.

From the data of Table 1, it can be seen that the ammoniacal form of nitrogen brings about an increase both in viscosity and in hydrophylic property of colloids in protoplasm, as well as in resistance to high temperatures. Along with this, resistance to low temperatures is decreased.

These facts agree with the data of the literature [4], as well as with our previous investigations [1], in which changes in plant resistance, depending on the colloidal-chemical state of their cells, were established. Along with this, one should note that the increase in heat resistance and simultaneous decrease in cold resistance which we observed in plants given the ammoniacal form of nitrogen do not support the statements of several authors concerning the existence of a single physiological resistance in plants.

We spoke above of the fact that the quality of nitrogen nutrition determines the rate of accumulation of organic acids in plant organs. In agreement with the data of Vladimirov [3], we also observed that the nitrate form of nitrogen sharply increases the rate of accumulation of organic acids in tobacco leaves (Table 1).

In connection with this, one can propose that the extensive accumulation of organic acids in tobacco leaves grown on nitrate nitrogen would have a definite effect on the viscosity of cell protoplasm.

However, the data of Table 1 indicate that the extensive accumulation of organic acids in leaves of tobacco given nitrate nitrogen does not have the expected effect on the changes of viscosity in cell protoplasm. This fact, from our point of view, is of definite interest, since it gives us occasion to suppose that the organic acids are not distributed in a diffuse fashion, but are localized in separate parts of the cell. In the case studied, the organic acids are evidently concentrated mostly not in the protoplasm, but in the vacuoles of cells.

TABLE 2

The Effect of Sodium Citrate and Sodium Oxalate on Viscosity, Elasticity, and Resistance to High Temperature of Protoplasm in Tobacco Leaves. (average of five determinations)

Source of nitrogen nutrition	Experimental treatments	Protoplasmic viscosity (time of transformation of plasmolysis, in minutes)	Protoplasmic elasticity (centrifugation time necessary for protoplasm separation, in minutes)	Heat resistance (temperature causing the death of cells)
$Ca(NO_3)_2$	Distilled water	35	5	44
	Sodium citrate	48	7	42
	Sodium oxalate	70	—	41
$(NH_4)_2SO_4$	Distilled water	50	10	46
	Sodium citrate	60	10	41
	Sodium oxalate	65	—	40

For checking this proposition, we have carried out a series of experiments in which tobacco leaves were saturated by 0.05 M solutions of sodium citrate and sodium oxalate (for an hour's duration).

After this, the viscosity and elasticity of protoplasm, as well as resistance to high temperature, were determined by the methods described above.

The results obtained are given in Table 2.

The data of Table 2 show that saturation of leaves with solutions of sodium citrate and sodium oxalate causes a noticeable increase in protoplasmic viscosity, and have practically no effect on protoplasmic elasticity. The increase of protoplasmic viscosity, in this case, we explain as a result of penetration of salts into the cell and their localization in the protoplasm. Thus, an important rule becomes clear, that in tobacco, during natural

synthesis, the organic acids are concentrated in the cell sap, while on artificial introduction of these substances into the cell, they are localized in the cell protoplasm.

The change of protoplasmic viscosity is reflected in its own way in the resistance of tobacco leaves to high temperatures. Sodium citrate and sodium oxalate cause a decrease in heat resistance, particularly in plants grown in the presence of ammoniacal nitrogen. Thus, the saturation of leaves by the salts mentioned above causes simultaneously an increase in protoplasmic viscosity and a decrease in the resistance of the plants to high temperatures. Consequently, the various sources of nitrogen have a substantial effect on the metabolism of the plants, which determines a certain difference in their reactions to factors of the external medium.

The present work was carried out under the direction of Professor P.A. Genkel', for which I should like to express my deep gratitude to him.

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THE EFFECT OF LOW TEMPERATURES ON EGGPLANT

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The attempt to increase the cold resistance of warm weather crops by cold treatment of their seeds prior to planting has attracted the attention of practical vegetable breeders for a long time. In recent years, Voronova [1, 2] has insistently recommended the hardening of seeds of warm weather plants by alternating temperatures. Also, papers of physiologists [3 - 9] began to appear, in which an attempt is made to understand the nature of the changes in the cooled seed and plants which cause further stimulation of growth and increase in cold resistance, or else injury and death.

The present work was carried out for three years in the Leningrad region, on the station of VIR. The main purpose of the work consisted in finding methods of obtaining considerable yields of eggplant in the open field in the zone which is north of their industrial cultivation, by studying the effect of low temperatures on eggplant at various periods of ontogenesis.

Experiment 1. The eggplant seeds, variety Maikopskii-15 were soaked in warm water and were kept at alternating temperatures, one group for 15 days, and another group for 30 days. Seeds were kept for 6 hours daily at a temperature of +25°, and for 18 hours at one of the following temperatures: -2°, 0°, +5° and +10°. Seeds of control plants were grown at a temperature of -25° for 7 days, which can provisionally be taken as the period during which the seeds could pass their stage of vernalization. The seeds of all treatments were planted on the same day – during the first days of April. Temperatures of -2°, 0°, +5° and +10° exclude the possibility of seed germination; consequently, the stage of vernalization cannot take place at these temperatures.

The best results, as indicated by growth, development and fruit yield in our experiment were shown by plants grown from seeds which were kept for 15 days at alternating daily temperatures (6 hours at +25°, and 18 hours at +5° and +10°). These plants bloomed 4 to 6 days earlier than the controls. A longer exposure (30 days) to the same temperature conditions causes a retardation of development and a decrease in yield (Table 1).

TABLE 1

Effect of Temperature Treatment of Seeds Prior to Planting on the Length of Vegetative Period and Fruit Yield of Eggplant

Seeds were kept for 6 hours a day at -25° and for 18 hours at t°C	Numbers of days of seed treatment	Number of days from germination to blooming	Weight of fruits, in g per plant
5	30	88	490
10	30	95	469
5	15	82	598
10	15	80	668
Control (24 hours at 25°)	7	86	549

As can be seen from Table 1, the yield of plants grown from seeds which were exposed to the action of alternating temperatures for 15 days exceeds that of the controls by 49 to 119 g. The retardation of plants and the decrease of fruit yield following prolonged treatment of seeds, we ascribe to their great sprouting (the sprouts reached a length of up to 1.5 cm).

Experiment II. In order to study the reaction of eggplant plants to changes in temperature conditions during various periods of ontogenesis, we carried out a laboratory experiment according to the following plan.

From the moment of planting, eggplants were grown in a hot-house for 10, 20, 30, 40, 50, and 60 days, and for the remaining period, until harvest, at a lower temperature. Vice-versa, other plants were grown for 10, 20, 30, 40, 50, and 60 days in the field, and for the rest of the time in the hothouse , at a temperature not lower than +20°. As controls, plants were used which were grown continuously (for 40 days) in the field or continuously in the hothouse . Plants of all treatments were grown on a short 12 hour day, and on a 24 hour day. The experiment was carried out with two varieties: Maikopskii-15 and Derbentskii-300. For the first 60 days in the hothouse, temperatures above 20° were prevalent, while in the field, temperatures varied between 15° and 20°. Such differences in the temperature regime had a noticeable effect on the development of the eggplants.

However, if plants were grown for 30 days at high temperature, and after that were transferred to the field, they bloomed simultaneously with control plants which were kept in the hothouse all the time. This indicates that eggplants react more positively to higher temperature in the beginning period of their ontogenesis. This is also indicated by the fact that plants which were kept at high temperature for only 10 days from the moment of germination bloomed on the sixty-third day, i.e. a week earlier than the controls which were kept in the field, which bloomed on the seventieth day. Consequently, high temperature is necessary for eggplant plants, beginning with germination.

The development of these plants is outlined in Table 2.

TABLE 2

The Time of Flowering of Eggplants in Relation to Temperature

Experimental treatments	Days prior to germination	Days from germination until flowering	
		Variety Maikopskii-15	Variety Derbentskii-300
Control (continuously in the hot-house)	10	56	63
Control (continuously in the field)	20	70	77
In the hot-house 10 days*	10	63	70
20	10	63	70
30	10	56	63
40	10	56	63
50	10	57	63
60	10	57	63
In the field 8 days**			
10	13	50	58
20	20	55	62
30	20	58	65
40	20	60	67
50	20	70	77
60	20	70	77

*The rest of the time until harvest – in the field.

**The rest of the time – in the hothouse.

One may believe that the stage of vernalization in eggplant is favored by high temperature, since eggplant is a southern plant in origin. At the same time, the data of Table 2 show that seeds planted in the field (at a temperature of 15° and lower), where they were kept for 10 days, and then were transferred to the hothouse, gave plants which developed faster than the controls (which were kept in the hot-house all the time) by 6 days in variety Maikopskii-15, and by 5 days in variety Derbentskii-300. Consequently, a short-term exposure of the sprouted seeds to lowered temperature, followed by high temperature, stimulates development. Such a result is similar to that produced by germinating seeds at alternating temperature. Evidently the reason for the development is the same in both cases.

We have also studied the effect of lowered soil temperature on growth and development of two varieties of eggplant – Maikopskii-15, and Skorospelyi VIR-061.

Dry seeds of these varieties were planted in metal containers of soil. From the moment of planting, the vessels were placed in a tank with running cold water (temperature 10°). The tank itself was placed in the hothouse, where the air temperature was from 18 to 22°. Control plants were grown continuously in the same hothouse. The first group of vessels was kept from the moment of planting for 5, 10, 15, 20, 25, 30, 35, 40, and 45 days at a soil temperature of 10°, and then these plants were grown together with the control plants at a temperature of from 18 to 22°.

The second group of plants was kept from the moment of planting for the same number of days at soil and air temperatures of from 18 to 22°, and then was placed into the tank with water (at 10°) for 45, 40, 35, 30, 25, 20, 15, 10, and 5 days. After 50 days, both control and experimental plants were planted in the open field, where they were grown until September 25. The experiment has shown that a temperature of 10° does not permit germination of the seeds. However, if the eggplant seeds were placed at that temperature for 5 to 15 days, and then were transferred to conditions of higher temperature (from 18 to 22°), they germinated on the third to seventh day, while seeds without previous cooling germinated on the tenth day. Seeds which were kept at 10° for not more than 15 days gave stronger, faster developing plants, which in the final account gave a higher yield of fruits, than those of the controls.

Thus, for example, plants grown from seeds which were kept for 5, 10, and 15 days at a soil temperature of 10° gave a higher yield of fruit than those of the controls, by 40, 32, and 27% respectively. The same soil temperature (10°) acts negatively on green plants. Thirty-five-day-old plants, given a soil temperature of 10° for 5 days, were retarded in their development by 3 days, and their yield of fruit was decreased by 13%; 30-day-old plants, grown at a soil temperature of 10° for 10 days, were retarded in their development by 17 days, and their yield of fruit was decreased by 47%, as compared to the controls. Therefore, the cooling of eggplant seeds at 5-10° for 10-15 days speeds up development and increases the yield of fruits.

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EFFECT OF GERMINATION CONDITIONS ON OXIDASES AND CATALASES OF RICE

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The reserve substances of endosperm undergo all kinds of transformations during germination of rice seeds. The main role in this process is played by respiratory enzymes [1], by means of which the embryo obtains energy for its living activity. As is known, rice is distinguished from other crops by the fact that its seeds are able to germinate at extremely low oxygen concentrations, or even without oxygen. This characteristic deserves attention, and elucidation of its physiological basis is of theoretical as well as of practical interest. The study of the chemistry of rice seed germination during submergence or wetting began a long time ago [2]. However, the investigations devoted to the study of hydrolytic enzymes are as yet insufficient for understanding the causes of the resistance of rice to anaerobic conditions. It is evident that, under these conditions, oxidative-reducing enzymes play an important role.

TABLE 1

Rate of Respiration (in μ liters of gas per g dry weight) and Respiratory Coefficient of Seeds and Seedlings under Various Conditions

Water regime	Seeds				Age of seedlings in days					
	Dry		Swollen		Just beginning to germinate		3-day-old		12-day-old	
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
	Respiration									
Wetting	0.27	0.35	36	70	58	112	651	651	1.085	1.085
Submergence	—	—	13	60	37	100	174	192	179	210
	Respiratory coefficient									
Wetting	1.14		1.82		1.80		0.60		0.60	
Submergence	1.48		4.60		2.94		1.20		1.18	

For the study of respiration and enzyme activity, an experiment was conducted in which seeds were germinated on filter paper, after wetting, or submerging them in a 15 cm layer of freshly boiled distilled water. The variety VROS 3716, belonging to the group of hygrohalophytes [2], and distinguished from other varieties by an extraordinary resistance to submergence, was used for the experiment. Respiration and the enzymes cytochrome oxidase and polyphenoloxidase were determined manometrically in a Warburg apparatus.

Catalase activity was also measured manometrically. Dehydrogenases were determined according to the method described by Gel'man [3].

The results of observations on respiration and on the respiratory coefficient are given in Table 1.

Respiration of germinating seeds is far more rapid in terms of oxygen absorption and carbon dioxide evolution in wetted seeds, as compared to those that are submerged. Together with this, our observations show that the respiration of wetted and submerged seeds is qualitatively different. When seeds were germinated on filter paper, a gradual increase in the respiration coefficient was observed, followed by a sharp decrease at the time of beginning of germination and further transformation of the embryo into a seedling. Evidently, the consumption of oxygen in oxidative processes begins at this period. In all probability, when seeds are germinated on a moist substrate, the anaerobic conditions for the growing embryo persist only for the first 1 - 2 days.

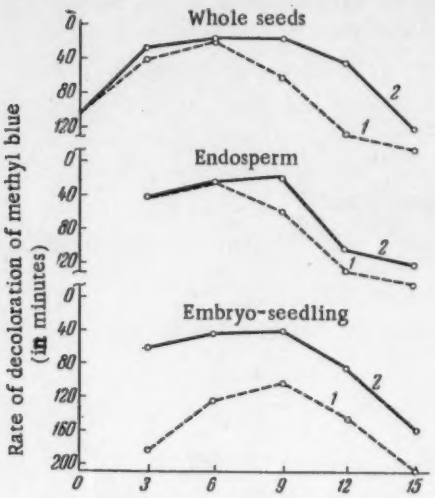

Fig. 1. Activity of dehydrogenases in germinating rice seeds. 1)Wetting; 2) submergence.

Germination in water is characterized by a value of the respiratory coefficient which is greater than one, while CO_2 production indicates that, during submergence, the main supply of energy for germination takes place in the first stage of respiration. In connection with this, the observations of dehydrogenases in the sprouting seeds are of interest (see Fig. 1).

Dehydrogenase activity in the endosperm is almost the same during wetting as in submergence, while in the embryo, and then in the seedling, it is considerably higher during submergence. In all probability, it is connected with the high physiological activity of growing leaves and rootlets.

The capacity of rice to germinate in the absence of oxygen is conditioned not only by the high activity of dehydrogenases under submergence conditions, but also by oxidative enzymes which may bind oxygen when it is present in extremely small concentrations in the surrounding medium [4].

The very high activity of catalase in the germinating rice seeds leads us to believe that such enzymes in rice germination are flavine oxidases. In order to check this hypothesis, it was necessary to determine the degree of participation of metal-containing oxidases in respiration of rice seedlings. For this, determinations of cytochrome oxidase and polyphenoloxidase have been made. The results of measurements at pH 6.81 and a temperature of 25° C are given in Table 2.

TABLE 2

Effect of Germination Conditions on Oxidases and Catalase of Rice at Different Seedling Ages (in days)

Water regime	1st	3rd	6th	9th	12th
Cytochrome oxidase •					
Submergence	0	0	0	0	0
Wetting	117.5	90.0	15.7	14.5	14.4
Polyphenoloxidase •					
Submergence	0	0	0	0	0
Wetting	0	0	73.5	99.4	133.4
Catalase activity ••					
Submergence	4.5	4.8	6.2	6.8	7.4
Wetting	12.5	45.5	58.3	128.9	145.4

• In μl O_2 per g wet weight per 30 minutes.
•• In ml per 20 kernels per 3 minutes.

Cytochrome oxidase activity is completely stopped during submergence. In seedlings on filter paper, its activity is relatively great during the first days of germination. With increase in age of seedlings, its activity decreases almost to one-tenth of the original value. The cytorchrome system is very sensitive to cyanides. However, cyanide poisons inhibit rice seedlings respiration only to 40% [5], while the rest is evidently accomplished by flavine oxidases, which are insensitive to cyanide. Polyphenoloxidase is also completely suppressed during submergence, while in the seedlings on filter paper, the polyphenoloxidase activity increases with age, in a direction opposite to cytochrome oxidase. It appears only on the sixth day after germination, when conditions become favorable for its appearance, in particular, when the substrates for its action begin to appear [5].

As is known, the polyphenoloxidase inhibitor is sodium diethyldithiocarbamate [6]. With the aid of the latter, it was possible to show that polyphenoloxidase

plays a very small role in rice respiration. The part it plays in respiration of rice seedlings at pH 6.81 and 25° can be seen from the following data. At the age of 6 days, the seedlings absorb 184 μl 0_2 per 30 minutes, while after inhibition with diethyldithiocarbamate, they absorb only 125 μl. At the age of 12 days, the corresponding amounts are 215 and 142 μl 0_2. Since the peaks of activity of various metal-containing oxidases appear in seedlings of various ages, since their values does not exceed 30-40% of the total respiration, it may be considered that the main part of rice respiration is accomplished by flavine enzymes.

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BURNS OF WHEAT LEAVES OCCURRING NEAR THE SOIL

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Under natural conditions often when plants are exposed to intense insolation they receive severe burns. These are accompanied by browning or yellowing of the leaves, twisting, and even death [1-6].

In addition to burns induced directly by high temperatures, there are also other kinds of burns which occur in nature. They occur when leaves of young plants come in contact with very hot soil. Such burns can be called soil surface burns to differentiate them from atmospheric burns induced by the direct effect of high temperature on the leaves.

Kobylin [8] was the first to observe soil surface burns on oat leaves, variety "Pobeda". While working at the Troitsky Steppe-Forest Game Reservation he observed wilting of oat shoots at an air temperature of 28° and a soil surface temperature of 46°. This wilting was associated with the formation of soil surface burns, or, as the author called them, the formation of dried rings.

We discovered soil surface burns on the fields in the Collective farm "Will of Lenin" at the Shadrinsk Experimental Station. They occurred even more intensively in the more southern areas of the Kurgan region. In 1955, at the end of May and the beginning of June, excessive heating of the soil was observed at the Shadrinsk Experimental Station. The temperature of the surface layer reached 58.5°. Leaves of wheat sprouts Liutestsens 758 which came in contact with the uppermost layer of the soil received severe burns; the younger the leaves, the more severely they were damaged.

The formation of transverse constrictions in the places where the leaves touched the soil was a characteristic sign of the soil surface burns. The burns had the appearance of a light yellow streak (constriction) which rose above the surface of the soil as the plants grew. New parts of young leaves which came in contact with the excessively hot soil also received burns. Some plants had as many as four or even five streaks. In the area of the burns the plants became bent over, as though broken, and gave the impression that the fields had been damaged by some unknown agent.

In order to determine the effect of soil temperature on the damage of young tissues, we made some daily observations on the number of injured plants as well as the depth of the injury.

Under field conditions it was difficult to determine which burn corresponded to which day. However, daily observations on the growth of one of these plants made it possible for us to determine without much difficulty which burn occurred on a particular day. Naturally, we didn't know what temperature existed during a particular time, but we did know the maximum temperature to which the soil was heated. But even this helped us to establish some sort of relationship between the heating of the soil and the damage to the plants.

We judged the degree of leaf injury by the area of the burned part as well as by the change in color of the leaf blade. We divided all the burns according to the degree of injury. We considered those plants in which only the margin of the leaf was damaged and the central part was intact as only weakly burned.

When the burns penetrated the entire leaf blade, the leaf injuries were more noticeable in those plants that had come in contact with the soil. Those plants in which a considerable area was burned had deeper injuries.

In relation to this the injury to the leaves could be classified into three categories: weak, severe, and very severe injury.

During the first days of June when the top layer of soil became heated to 33.6 - 58.5°, various degrees of plant injury were observed.

Comparing the data obtained for burns on the plants with the data concerning maximum soil temperature, one can conclude that there was a direct relationship between soil temperature and injury to the tissues of young plants: the higher the soil temperature and the longer this high temperature lasted, the more severe the degree of burning (Table 1).

The greatest number of very severely injured plants occurred at soil temperatures of 54.5 - 58.5° and air temperatures of 32.8 - 38.4°. (June 3, 6, 10, 18, 19, 20). With a decrease in air temperature, which also resulted in a decrease of soil temperature, the number of very severely injured plants decreased considerably. When the soil temperature decreased from 58.5° to 53.1°, the number of very severely injured plants decreased from 298 to 82, but this was still high. At still lower soil temperatures (June 9, 12, 13, 17) the number of very severely and severely injured plants decreased sharply, whereas the number of weakly injured plants increased.

TABLE 1

Degree of Plant Injury Caused by High Soil and Air Temperatures*

Date (June 1955)	Number of leaves	Maximum soil surface temperature	Weather conditions			Number of plants injured by high temperature			
			Maximum air temp., in°	Relative humidity of the air in %	Wind velocity, in m/sec	Weak	Severe	Very severe	Total
1	1	51.8	35.2	42	2.7	62	118	12	192
2	1	53.1	35.5	44	3.5	62	115	82	259
3	1	55.3	37.3	34	2.7	4	122	274	400
9	2—3	50.3	29.7	52	3.0	86	74	1	161
10	2—3	54.5	32.8	37	3.0	38	144	132	314
11	3	45.3	29.2	87	0.9	2	0	0	2
12	3	49.6	31.4	64	2.8	89	22	0	111
13	3	54.5	36.3	36	2.6	49	124	144	317
14	3	49.3	34.2	52	3.2	71	26	0	97
16	4	46.1	24.6	56	2.1	4	0	0	4
17	4—5	51.5	28.9	46	3.2	73	126	4	203
18	4—5	55.0	36.7	42	2.3	12	184	164	360
19	5	58.5	37.4	42	3.2	24	181	298	503
20	5	57 6	38.4	39	3.1	16	176	253	445

* The soil temperature was calculated from the data obtained by the Shadrinsk Weather Station. It was measured in wheat stands in the usual pairs. The other figures (air temperature, relative humidity, and wind velocity) were calculated on the basis of our own measurements which were made at the height of the plants at 1:00 p.m.

From this it appears that soil temperature above 54.5° induced severe soil surface burns. These plants were bent in the burned areas as though they had been broken. In some cases part of the leaf above the burn lost its turgor. At a soil temperature from 50.3 to 53.1° and an air temperature from 29.7 to 35.5° the plants also had soil surface burns, but they were not so penetrating. The injured plants were bent in the burned area, but a loss of turgor was not observed. At a soil temperature of 49-50° the plants were less severely burned. As a rule they had some hardly noticeable burns at the leaf edges. Along with this, after 5-6 days the burns were not even noticeable in some of the plants. Apparently partial or complete tissue regeneration occurred after such burns.

The data concerning the viability of cells after various degrees of burning are of certain interest. The viability of the cells was determined by two different methods: the plasmolytic method and vital staining with

neutral red. Data obtained by the plasmolytic method showed that plasmolysis was observed in the uninjured cells within 3-4 min. No plasmolysis at all was observed after severe or very severe burning. This indicates that the cells in severely injured plants were either partially or wholly dead. It was characteristic that the viscosity of the protoplasm increased noticeably in the cells injured by high temperature. The viscosity of the protoplasm which was determined plasmolytically, in the uninjured cells remained the same for 9-10 min. In cells which had been injured by high temperature it increased 5-6 times.

Similar results were obtained using the vital staining method. Sections of the upper and lower epidermis were immersed in neutral red (1:5000) for 10 min. After this elapse of time the sections were rinsed with water and studied under the microscope. In the control cells (uninjured) the dye was concentrated in the vacuole; it stained neither the nucleus nor the other protoplasmic inclusions. The results were completely different for the cells injured by high temperature. In the severely and very severely injured cells the vacuole remained unstained. The nuclei or the cells stained a deep red. The protoplasm also became stained a brick-yellow. No difference was observed between the cells which had been only slightly injured and those from the control plants. However, they differed from the controls in that the dye in the vacuole became granulated very rapidly (in 15-20 min), whereas in the controls the dye stained the cell sap evenly, and the dye became granulated only after 50-70 min. This fact is evidence that one of the very primary disruptions is the disruption in the osmotic activity of the cell.

Hence both methods revealed that during slight over-heating part of the cell function was disrupted although the cell continued to live. During severe burning irreversible protoplasmic injury occurred which resulted in the death of the cells.

There is some indication in the literature that wheat belongs to the group of plants which are distinguished by a low temperature optimum, especially during its early phases of growth. High temperatures, according to Dickson [9], bring about severe injury, and according to Tottingham [10] and Hurd-Darrer [11] – to an impoverishment of the vegetative parts, reduction of stooling, and reproduction. The data obtained by the authors agrees completely with the laboratory investigations by P.A. Genkel' concerning the lower heat resistance of plants during their early stages of development in relation to low viscosity of their protoplasm.

Consequently, during the early phases of development wheat suffers severely from soil surface burns. The burns occur when the protective covering of the leaf (epidermis and cuticle layers) has not been completely formed.

Very high soil temperatures (higher than 54°) produced very severe burns in wheat plants. Plants also received burns when the soil temperatures ranged from 51 to 53°, but these were less severe. Soil temperatures below 50° produced slight burns which were hardly noticeable at the leaf edges.

As a consequence of slight soil surface burns part of the vital functions of the cells were destroyed, however the cells apparently continued to live. Severe burns brought about an irreversible injury to the protoplasm which resulted in the death of the cell.

In conclusion the author wishes to express his sincere appreciation to Prof. P.A. Genkel' who directed the work.

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SOME PECULIARITIES OF WATER RELATIONS IN ONE-AND TWO-YEAR-OLD ELM AND ENGLISH OAK SAPLINGS

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Experiments on the water relations of English oak, elm, and several other species of trees and shrubs performed by one of us in the arid regions of Stalingrad [1, 2] established that English oak uses water considerably more economically than other woody species. In all the observations the transpiration rate of this species was lower than that in English elm, Turkestan elm, green ash, and other species. It was also noticeable that the curve for the transpiration rate followed the changes in the intensity of meteorological factors only when the soil water content was high. When the soil was dry the curve for the rate of transpiration deviated from the typical course and the maximum rate occurred in the early morning hours. Ivanov et al. [3], Khlebnikova [4, 5], Tsel'niker [6], and others arrived at the same conclusion in their investigations.

We undertook the present investigation using one- and two-year-old saplings under propagating conditions in order to check the conclusions which we had established for mature plants. The experiments were begun in the summer of 1952 and 1953 in the propagation house of the plant physiology department at the Moscow State University in Moscow. The plants were grown in soil cultures at soil moistures of 80, 60, or 40% of full moisture capacity.

The Effect of Soil Moisture on the Transpiration Rate and Stomatal Opening of One-Year-Old English Oak and English Elm Saplings

E. Hoehnel was the first to begin a study of transpiration in woody plants at various soil moisture conditions. He worked primarily with five-year-old saplings of woody species rooted in large propagating containers. He showed that when the moisture content of the soil decreased, the transpiration of water from the plant also decreased.

In order to measure the rate of transpiration we weighed the containers with the plants every 3 hours throughout the experiment. Evaporation from the soil surface was prevented by covering the surface with waxed paper. At the same time observations were also made on the conditions of the stomates. From Table 1 it is evident that the higher rates of transpiration in two-year-old saplings of both oak and elm were observed in plants grown at higher soil moistures.

The daily fluctuation in transpiration rate in both woody species was due to the changes in weather conditions (Table 2). We also observed the same relationship in an experiment with one-year-old saplings of the same species which we had performed on July 27, 1953. In many cases we were unable to observe a complete agreement between the degree of stomatal opening and the transpiration rate. For example, the highest rate of transpiration in both species was observed at a soil moisture of 80% full moisture capacity, whereas the stomates were opened the least at this soil moisture (Table 3).

However, in some cases there was a fairly good agreement between the opening of the stomate and the changes in transpiration rate. For example, in elm at soil moistures of 40 and 60% there was a close relationship between the change in transpiration rate and the change in size of the stomatal aperature. Substantial variations were observed between two-year-old saplings of these two species. The transpiration rate of elm was usually higher than that of oak (Table 1). Such large variations were also observed in the degree of stomatal opening in

TABLE 1

The Effect of Soil Moisture on the Transpiration Rate of Two-Year-Old English Oak and English Elm Saplings

Name of plant	Soil moisture in % of full moisture capacity	Leaf area in dm^2	Transpiration rate in g/dm^2 per hr.				
			7:00-10:00	10:00-1:00	1:00-4:00	4:00-7:00	average per day
English elm	80	22.31	1.19	1.84	2.02	1.12	1.54
	60	21.87	1.22	1.52	1.52	0.87	1.28
	40	16.80	1.29	1.54	1.68	0.79	1.32
English oak	80	15.6	1.03	1.09	1.28	0.42	0.93
	60	15.3	0.76	0.94	0.98	0.33	0.78
	40	10.8	0.61	0.93	1.05	0.36	0.74

TABLE 2

Daily Variations of Meteorological Factors

Meteorological factors	7:00 A.M.	10:00 A.M.	1:00 P.M.	4:00 P.M.	7:00 P.M.
Air temperature, in °C	21.0	22.5	24.5	24.7	24.3
Relative humidity of the air, in %	82	74	68	61	60
Cloudiness, in points	0.1	3	5	3	4
Wind	None	Weak	Weak	Moderate	Weak

oak and elm (Table 3). Throughout the entire day the size of the stomatal opening was usually larger in elm than it was in English oak. Also during the day where was a greater number of closed stomates in oak than in elm.

TABLE 3

The Effect of Soil Moisture on the Width of the Stomatal Opening in Two-Year-Old English Elm and English Oak Saplings

Name of plant	Soil moisture in % of full moisture capacity	Width of stomatal openings in microns at the given hours						Percentage of closed stomates at the given hours				
		7:00 A.M.	10:00 A.M.	1:00 P.M.	4:00 P.M.	7:00 P.M.	daily average	7:00 A.M.	10:00 A.M.	1:00 P.M.	4:00 P.M.	7:00 P.M.
English elm	80	0.98	0.39	1.41	1.88	0.164	0.966	10.7	40.2	4.33	0	68.0
	60	2.87	1.91	2.46	2.07	0.404	1.943	0	0	0	0	41.5
	40	2.29	2.04	2.82	2.39	0.450	1.997	0	0	0	0	13.2
English oak	80	0.76	0.384	0.49	0.40	0.028	0.508	0.98	15.08	1.01	23.5	92.9
	60	0.31	0.487	0.61	0.22	0.005	0.406	35.56	7.55	0	51.8	87.5
	40	0.62	0.496	0.64	0.07	0.016	0.369	11.10	4.45	0	81.8	90.5

In elm stomatal closing usually began 3 hours later than it did in English oak; hence the stomates in elm were open for a greater number of hours.

The Effect of Soil Moisture on the Water Deficit and on the Total Water Content of the Leaves

In addition to the rate of transpiration we also studied the water content of the leaves. Leaf samples were picked at 10:00 a.m. on July 12, July 25, August 23, and September 8. The results of these measurements are given in Table 3. In both species the water content of the leaves decreased as the soil moisture decreased.

Oak leaves, as a rule, contained less water than those of elm.

Oak and elm differed not only in the total water content of the leaves, but also in the degree to which the tissues were saturated with water. The moisture capacity of oak, as well as elm leaves, (water content during complete saturation) decreased when the soil moisture decreased. The water capacity of elm leaves was considerably higher than that of English oak leaves.

As the soil moisture decreased the water deficit of the leaves, or the diffusion pressure deficit, increased.

Elm and oak also differed from each other in the magnitude of their water deficit: in elm it was considerably higher and fluctuated between 16.9-21.5%, in oak these fluctuations did not exceed 5.7-10.7%.

As one can see from this data, there was a decrease in the transpiration rate of both species when the soil moisture decreased; we had also noticed this in mature plants at the arid conditions prevailing around Stalingrad.

Our results agree completely with those obtained by Höehnel [7]. However, in our experiments which were performed under conditions prevailing around Moscow not once did we get curves for transpiration rate with the maximum value deflected to the early morning hours during a low soil moisture content. We had usually obtained such curves during drought periods around Stalingrad. In our experiments performed in the environs of Moscow the maximum transpiration rate of both species grown at soil moistures of 80, 60, and 40% full moisture capacity always occurred at the time of the greatest stress in weather conditions.

The transpiration rate in all the variants of the experiment was lower in English oak than in elm, just as we had observed in the Stalingrad region.

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TRANSPIRATION OF ACCRETE PINE TREES

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Instances of roots, root crowns, stems, and branches of woody species coalescing have been described and studied by many Soviet and foreign investigators. According to N.P. Krenke "Coalescence occurs when two or more living organs which have arisen individually form a physiologically interacting, structurally inseparable unit, without injury, by means of the accretion or 'resorption' of the elements in at least one of them" [1]. Expressed in other words coalescence is identified with "self-grafting" or "natural grafting".

The work of several Soviat investigators has shown that the coalescence of woody varieties within a species increases their vitality, resistance and productive capacity.

Nikitin [2] considered the coalescence of woody varieties within a species as a natural intraspecies type of vegetative hybridization.

We studied the coalescence of root systems and the process of biogroup formation in experimental plantings at the Kamyshinsky forest improvement developing area of the All-Union Scientific Experimental Institute of Forestry Improvement (VNIALMI) for four years. The work was done in pure and mixed stands of 35-48 year-old pine trees growing in chestnut soils covered with a layer of sand to a depth of 0.3 to 1.0 m.

It has been established that in pure stands of 35-year-old pine trees about 50% of all the trees belong to biogroups formed as a result of the coalescence of their roots. Biogroups usually consist of 2-6 trees, but certain biogroups of 10-12 trees have been reported. If we take the volume of an average individual tree as 100%, then the volume of an average tree in a biogroup of two trees would be 174% in a biogroup of three trees, 170% in one of four trees, 181% in one of 11 trees, 263% [3].

In the meantime, differentiation of the trees in the biogroup is more distinct than in the individual ones. Because the gradual physiological interaction between the trees within the biogroup (through the site of coalescence) we can consider the biogroup as a colonial organism, and the individual trees as parts of this organism. Usually when several trees which are almost identical in their growth grow together, even in the biogroup they develop almost identically in comparison with each other. However, when one of the coalescing plants lags behind the others in its size, then after coalescence these "lagging"components frequently slow down in growth without, however, displaying any symptoms of oppression. They form a better developed, thick, low crown. Apparently these trees obtain additional materials from the taller trees whose crowns are located in more favorable light conditions.

In some cases when several trees located very close to each other coalesce, and one of them has been considerably smaller than the others, it ceases growing in height completely, forms weeping branches which touch the soil, and a severly bent trunk. Such characteristics of development among the trees of a biogroup indicates that the physiological processes in the various trees in the biogroup are not identical and differ from the physiological processes in the individual uncoalesced trees.

In the summer of 1955 we made a comparative study of the daily course of transpiration in trees within biogroups, and in trees that had not coalesced within pine stands described above. This was done by means of rapid weighing. The transpiration chamber was mounted on two wheels; this made it considerably easier to move it from tree to tree. The weighings were made in five replicates, correct to the nearest mg. The exposure was

Fig. 1. Biogroup of three coalesced pine trees.

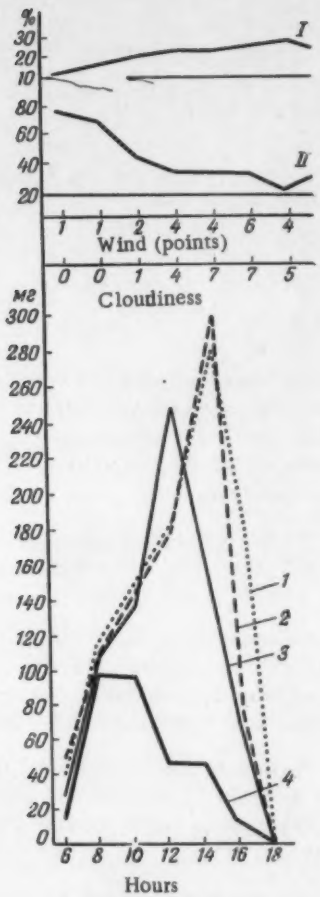

Fig. 2. Curves for the course of daily transpiration (in mg per dm^2 per hr) in coalesced pines and control trees. 1,2,3) The number of the tree in the biogroup; 4) control tree; I) air temperature; II) relative humidity of the air.

3 min. The loss of water was calculated per unit surface area of the needles. The surface area of the needles was determined according to Tiren's formula where the cross section of the needle is considered as a polyellipsoid.

$$P = \frac{\pi}{2} D\ (1.37\ V + Ch)\ 0.9$$

where P is the surface of the needle, D is the length of the needle, V is the breadth at the middle and Ch is the depth at the middle.

In view of the fact that the needle is not of the same breadth and depth throughout its length, the author introduced a correction factor, 0.9. For more pointed needles the correction factor used was 0.8. The breadth and depth of the needle was measured with a microscale to the nearest 0.01 mm.

The surface are of 20 needles picked from the same part of the tree from which the branches for the experiment were picked, was measured for each tree. Following this the area of 1 g of needles for a given tree was calculated and this value was used in later calculations.

We studied the course of daily transpiration within a biogroup consisting of three coalesced pine trees shown in Fig. 1. The dimensions of these pines are given in the table.

As we see from the table the volume of the average tree of the biogroup was more than two times greater than the volume of a single tree.

The nature of the daily course of transpiration in the trees of this biogroup can be judged from Fig. 2. An analysis of the curves shows that the magnitude of transpiration did not exceed 100 mg per 1 dm^2 per hour in the individual pine trees. At 10:00 a.m. when the relative humidity of the air dropped to 45% m and the temperature

TABLE

Compounds	Height, in m	Diameter of root crown in cm	Diameter at a ht. of 1.3 m, in cm	Growth in height, in cm	Volume, in m^3	Average volume of tree in biogroup
1	9.0	38	27/23	8	0.420	0.200
2	8.7	27	23	7	0.171	
3	4.0	14	9	—	0.011	
Control	5.9	25	20	1	0.094	0.094

had risen to 20°, a sharp decrease in transpiration occurred with continued until transpiration ceased completely. Consequently, the tree transpired very weakly during the hottest part of the day. This apparently occurs because by 10:00 a.m. a large part of the water absorbed by the tree during the night has already transpired. Insufficient water during the hot hours of the day is not beneficial to the tree since a decrease in transpiration leads to the decline of many of the life processes of the organism, and consequently to a decrease of resistance and productive capacity.

Coalesced pines transpired at a considerably higher rate than individual ones (see Fig. 2), whereupon as the air temperature increased and the relative humidity decreased the magnitude of transpiration did not decline as it did in the control, but in increased. The greatest value occurred at 2:00 p.m. (300 mg per dm^2 per hr) i.e. three times greater than the control tree. After 2:00 p.m. a sharp decrease in transpiration was observed in trees No. 1 and No. 2., whereas this decline in tree No. 3 occurred after 12:00 o'clock.

Since tree No. 3 was shaded by trees No. 1 and 2 it suffered less from the excess heat of the sun. Therefore we can conclude that during an inadequate supply of water some water passed through the site of coalescence from tree No. 3 to trees No. 1 and 2 where the need for it was greater.

The more intense transpiration of the coalesced trees indicates their greater resistance to drought and their better adaptability to the severe conditions of forest growth. As early as 1915 Maximov [4] showed that xerophytes can not be considered as plants with a low transpiration rate and good water retention, and that many xerophytes are characterized by a high transpiration rate.

According to N.A. Maximov, the ability of plants to withstand drought without the cells losing their vitality when their water content is low is a primary characteristic of drought resistance in plants. Similar results were obtained later by Vasil'ev (1927, 1931), Kuzmin (1930), and also several other authors whose results Razdorsky refers to in his book (1949).

Hence, the nature and magnitude of transpiration in the coalesced and individual trees varied greatly. Therefore, during a study of transpiration in woody species it is essential to take into account the effect of coalescence of the root systems of the trees, to find out the size and number of biogroup formed as a result of this, the percentage of coalesced and single trees in the stand, etc. Transpiration should be studied separately in the biogroup as well as in the individual trees.

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GRAVITATION OF THE EARTH AS A FACTOR IN THE FORMATION OF PLANT ORGANS

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The evolution of plant organs occurred during a constant exposure to the force of the earth's gravity. It is, therefore, quite natural that this factor of the external environment should have left a specific imprint on the activity of the plant organs and become an essential factor in their growth and development.

All the growth processes in a plant are to some degree or other counter balanced in respect to the gravitational force of the earth.

It is known that a quantitative or directional change in the force of gravity results in a significant shift of the growth processes which affect the formation of organs and their rate of growth.

G.Kh. Molotkovskii's experiments [1] have established that a direct reversal of the orientation of the plant with the apical end downward inhibits the formation of reproductive organs, displaces the budding zone, etc. In the experiments by Hes [2], centrifuging stem cuttings of sugar cane with the basal end outward, accelerated the sprouting of the buds at the apical end considerably. Whereupon centrifuging the cuttings with the apical end directed outward strongly inhibited sprouting of the buds at the apical end basal end of the cutting as compared with the controls. Jones [3] was able to induce the formation of stem buds and chlorophyll at the basal end by centrifuging root cuttings of Crambie maritima. The change in the correlating relationship due to gravitational force wtill remains inadequately studied regardless of the abundance of investigations concerned with this problem. The mechanism of the effect of gravity is still completely vague. Centrifuging sea urchin eggs with a force exceeding the gravitational force of the earth by 150,000 times does not result in visible, morphological changes of the contained protoplasm [4]. At the same time, at this velocity of centrifuging Svedberg was able to separate proteins in vitro. Apparently in living systems the forces which maintain their colloids in a certain conditions are very great, and consequently they either change very little or not at all as the result of application of gravitational force. Therefore, centrifuging living colloidal systems does not result in a dissociation of proteins according to their molecular weight, but it does substantially change their functioning.

Gravity, together with other factors, in all probability has a great effect on the distribution of various metabolites as well as substances with a high physiological activity among the various organs and parts of plants.

Considering the morphological polarity of a plant as the appearance of polarity in the structure of the protoplasm, one might conclude that centrifuging the entire plant or else parts of it results in the redistribution of metabolites, and as a result a change in the morphological polarity. In relation to this we used the centrifuging method as a means of disrupting the correlation between the plant organs.

Establishing the possible role that gravity plays in life processes of a plant organism is of great theoretical interest from the view point of general biology, but it is also of practical importance in connection with an effective development of investigations concerned with cosmic growth. In that case we will have to deal with another numerical value for gravity as compared with its value on earth; therefore a study of the effect of gravity on the life processes of plants now becomes a real problem.

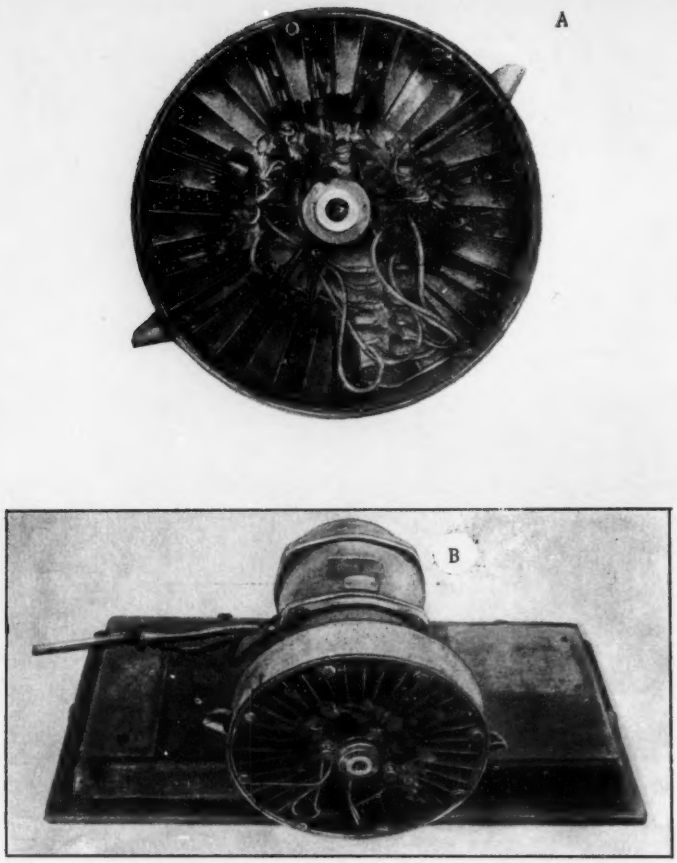

Fig. 1. An apparatus designed to apply a centrifugal force to cuttings exceeding the gravitational force of the earth by 256 times (A) and the same apparatus attached to an electric motor (B).

In the present investigation our object was to disclose the effect which a change in gravitational force has on the shifting of the polarity of organ formation in black raspberry cuttings.

In order to perform these experiments we constructed a special apparatus which made it possible to apply to the cuttings a centrifugal force exceeding the gravitational force of the earth by 256 times (Fig. 1). The apparatus consisted of a round chamber that could be closed, and which was firmly fastened to the horizontal shaft of an electric motor. The inside of the chamber was divided into 17 sections. The cuttings were fastened in these sections with rubber stoppers. Knop's nutrient solution was poured into the chamber; as the chamber turned the solution was distributed evenly around the walls. The cuttings were thus immersed in the solution to one-third of their length. As the chamber turned air moved continuously into each section. In order to supply air to the side walls of the chamber two vents were made opposite each other; these ended in tubes which reached into the central cavity of the chamber. Special fans were fastened on the outside near the vents; one fan served to force the air into the vent, and the fan near the opposite vent helped to evacuate the air from the central cavity of the chamber when it was rotating. A manifold was attached by means of a rubber tube to the tube leading from the intake vent. From the manifold air entered into each section of the chamber through slender rubber tubing. The air passed through the solution in each section, into the area of the chamber without nutrient solution, and then was ejected through the tube of the other vent. Automatic aeration of the nutrient solution was thus accomplished continuously throughout the day during the entire exposure period. The front lid of the chamber was made of

clear plastic; this made it possible to illuminate the cuttings throughout the entire experimental period. The chamber was filled with cuttings through the front lid which was removable and was attached to the back lid of the chamber with eight bolts. Rubber or leather gaskets were placed between each separate part of the chamber; this made it possible to seal each of them tightly and prevent the loss of nutrient solution from the chamber when it was rotating. There were two holes with lids in the front cover of the chamber; these were used to introduce the nutrient solution. The solution was poured in after the chamber was filled with cuttings and sealed tightly.

Fig. 2. Raspberry cuttings. A) arranged in the chamber with the apical ends to the center, and B) with the basal ends to the center; C) controls in a moist chamber arranged normally, and D) inverted.

Black raspberry cuttings were placed into the chamber with either their apical end (Fig. 2, A) or their basal end (Fig. 2, B) toward the center of rotation. The exposure period was exactly 43 days (April 2 - May 15, 1957). A control was set up at the same time in a moist chamber with the cuttings placed either normally (Fig. 2, C) or up-side-down (Fig. 2, D). The cuttings which were rooted in a normal position (Fig. 2, C) developed callus and formed roots at the basal end of the cutting, as usual. In the inverted position (Fig. 2, D) callus developed partially without the formation of roots. Some depression was observed in the cuttings which were in an inverted position throughout the entire experiment.

When centrifugal force was applied, the cuttings which were placed with their apical ends toward the center of rotation developed callus and roots at the basal end, and shoots and leaves at apical end.

A comparison of the cuttings which rooted in a normal position with the application of additional gravitational force (Fig. 2, A) and those without it (Fig. 2, C) made it possible to note the following differences: in the centrifuged cuttings the roots developed near the cut surface and almost always arose from callus. With the application of centrifugal force the zone of root formation appeared displaced to the very end of the cutting. The leaves and shoots of these cuttings were well developed, however they were turned toward the basal end, i.e. in the opposite direction from the center of rotation. The roots on the cuttings which were not centrifuged (the controls) were distributed loosely at the basal end and covered $\frac{1}{4}$ to $\frac{1}{5}$ of the length of the cutting. In this case

considerably fewer roots appeared directly from the callus zone. The leaves and shoots of these cuttings were less developed.

The cuttings which were centrifuged in an inverted position (Fig. 2, B), i.e. with the basal end placed toward the center of rotation, disclosed a very interesting picture. On these cuttings callus formation was observed at the basal end, and root formation at the apical end, i.e. there was a space interval between the formation of callus and roots. The leaf blades were less developed in these cuttings than in those not inverted.

From these experiments we can conclude that by increasing the gravitational force considerably one can change the morphological polarity of a plant. Furthermore, callus formation is not always a preliminary stage of root formation. Roots and callus may develop independently of each other as two parallel processes. In order for roots to develop it is necessary to have a movement of certain metabolites toward their site of formation; this apparently also plays a part in the formation of roots at the apical end during centrifuging. The redistribution of metabolites in plants may play a role in the intensity of the action of various factors (chemical, physical, etc.), and among them, evidently, the change in force of gravity. The force of gravity is, therefore, one of the many factors which has an effect on the appearance of polar characteristics of the organism. Establishing the role of this factor in the life activity of plants and discovering the conditions which strengthen or weaken the manifestation of the gravitational force is an important theoretical and practical problem.

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THE ABILITY OF WHEAT COLEOPTILE TISSUES TO HYDROLYZE CERTAIN SUBSTITUTED AMIDES OF 2,4,5-TRICHLORPHENOXYACETIC ACID

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It has been assumed that the physiological activity of herbicides belonging to the group of halogen derivatives of phenoxyacetic acid is associated with the presence of a carboxyl group within the molecules. Therefore the activity of ethers, amides, and nitriles of these acids depends on the ability of the plant tissues to hydrolyze them [1].

As a result of experiments on tomato with 2,4-dichlorophenoxyacetyl derivatives of amino acids Wood and Fontaine [2] came to the conclusion that plants hydrolyze derivatives of l-amino acids but are not capable of dissociating derivatives of d-amino acids. A study of the effect of amino acid derivatives of other halogenated phenoxyacetic acids and 2,4-dichlorophenoxy-α-propionic acid on other plant species [3-5] revealed that in some cases derivatives of d-amino acids also have a herbicidal effect. Therefore it is assumed that the plants are able to form derivatives of l-amino acids from corresponding derivatives of d-acids. Krewson, et al., [4, 5] also considered that the activity of amino acids from derivatives of halogenated phenoxyacetic acids depends on the structure and physicochemical properties of the entire molecule as a whole.

TABLE 1

The Effect of Potassium Salts of 2,4,5-T, l-TG, and d-TG on the Growth of Wheat Coleoptiles

Potassium Salts	Concentration, in mg/l	Length of coleoptile, in % of control after treatment for			
		24 hr	48 hr	72 hr	83 hr
2,4,5-T	10	106.1	134.0	161.6	157.0
TAB	14.7	106.7	133.0	149.5	162.8
l-TG	15.2	104.2	134.7	164.0	175.1
d-TG	15.2	104.9	133.2	166.0	164.0
Water (control)	–	102.7	113.2	135.0	114.2
2,4,5-T	1000	–	–	105.1	120.8
TAB (triethyl ammonium salt)	1655	–	–	138.8	146.8
l-TG	1520	–	–	101.7	121.3
d-TG	1520	–	–	114.8	124.1
Water	–	–	–	131.6	148.9

There is no direct evidence concerning the hydrolytic dissociation of amides from halogenated phenoxyacetic acids by plant enzymes, or else from direct data, to support this hypothesis.

We performed an experiment to ascertain the formation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by tissues of wheat coleoptile treated with N-2,4,5-trichlorophenoxyacetyl-n-aminobenzoic acid (TAB), N-2,4,5-trichlorphenoxyacetyl-l-glutamic acid (l-TG), or N-2,4,5-trichlorophenoxyacetyl-d-glutamic (d-TG) acid.

The work was done essentially according to the method described by Wain and Wightman [6], who studied the formation of 2,4,5-T during the β-oxidation of 2,4,5-trichlorophenoxy-γ-butyric acid by wheat tissues.

The substance being studied were prepared as follows: 2,4,5-T, (melting point 153°) was prepared by repeated recrystallization of the commercial product from dichlorethane and ethyl alcohol. TAB (melting point 289-290°) was synthesized by the interaction of the chloride of 2,4,5-T with p-amino-benzoic acid and was purified by recrystallization from dimethyl formamide.

l-TG and d-TG (melting point 123-124°) were obtained by the method described [7] and were purified by recrystallization from benzene and dioxane, and the separation of the acid solution from a mixture of benzene with an acetone of petroleum ether.

Salts of 2,4,5-T, TAB, l-TG, and d-TG were prepared by evaporating to dryness water solutions obtained by mixing water suspensions of the corresponding acids with an equivalent amount of base. In order to prepare the solutions we dissolved weighed portions of the salts 2,4,5-T (10 mg and 1000 mg), TAB (potassium salt 14.7 mg, triethylammonium salt 1655 mg), l-TG and d-TG (14.7 mg and 1470 mg) in an adequate amount of boiling distilled water and the solutions were brought up to 1 liter in a volumetric flask. In both instances the salt solutions of TAB were cloudy. Moreover, less concentrated solutions (first series of the experiment) of an equimolecular concentration of the potassium salt of 2,4,5-T (10 mg/l) were stimulating, and solutions of a higher concentration (second series of the experiment) of an equimolecular concentration of this compound were inhibiting (1000 mg/l).

One might conclude that when the salt was dissolved in hot water there was a partial hydrolysis of the amides with a separation of 2,4,5-T. However chromatographic checks revealed that 2,4,5-T was not present in salt solutions of TAB, l-TG, and d-TG.

Wheat coleoptiles were grown and prepared according to Boiarkin [8].

One hundred wheat coleoptile sections, variety Liutestsens 230, 10 mm in length were placed in Petri dishes containing 25 ml of solution. For the control 100 coleoptile sections were placed in a Petri dish containing 25 ml of water. The Petri dishes set up for the experiment were placed in an incubator for 30 min., 24, 48, 72, and 83 hours. After the specific time had elapsed the coleoptile sections were removed from the dishes and their length measured.

The data obtained for the growth of wheat coleoptile sections treated with the experimental compounds (Table 1) shows that at a concentration of 10 mg/l all the compounds stimulated growth, whereas at concentrations 100 times greater 2,4,5-T, l-TG, and d-TG inhibited growth of the coleoptiles, and TAB was inactive.

The composition of the solutions with which the coleoptiles were treated was determined by the use of chromatographic paper. Each solution was acidified with 0.05 ml 2 N H_2SO_4 at the stimulating concentration of the salts and 5 ml of acid when the concentration was inhibiting, and extracted with distilled ethyl ether three times (25 ml ether for each extraction). Furthermore, because TAB did not dissolve sufficiently in ether it was not extracted completely, but only in an amount adequate for a chromatographic determination.

The ether extracts were dried with annealed $MgSO_4$ and the solvent was distilled off. In the first series the dry residue was dissolved in 0.15 ml dimethylformamide, and in the second series in 15 ml of dimethyl formamide; 0.05 ml portions of the obtained solutions were placed on chromatographic paper 52 cm x 18 cm large. The spots obtained contained 80-125 μg of the extracted compound. Chromatographic paper used in the investigation was obtained from the Volodarsky Leningrad factory No. 2 (slow). In order to separate the materials the descending chromatography method was used. The chromatograms were placed in cylindrical chambers with the solvent. A mixture of 836.8 ml butyl alcohol, 24.7 ml water solution of ammonia (d 0.898), and 138.4 ml of water was used as the solvent. In addition to the extracted compounds, 0.05 ml of solution of the corresponding spots in dimethylformamide (10 mg acid dissolved in 5 ml dimethylformamide) were also placed on the paper. Fractional separation in the solvent lasted 21 hours. The chromatograms were dried in the air and then were examined first in ultraviolet light (Ultrachemiscope I), before they were developed with a solution of bromcresol green (0.1 g of the indicator dissolved in 100 ml water containing 2.9 ml of 0.05 N NaOH), or a 1% water solution of litmus. The location of dark spots of TAB were noticed in the ultraviolet light on a violet background. 2,4,5-T, l-TG and d-TG produced less noticeable spots. Yellow spots of 2,4,5-T, l-TG and d-TG on a blue-green background were detected when the chromatogram was moistened with a solution of bromcresol green. Less noticeable spots appeared when the chromatogram was moistened with litmus. In all instances the distribution of the spots corresponded to the location of the test spots. Sketches of the chromatograms are given in the figure. From these it is evident that wheat coleoptile sections treated with stimulating doses of l-TG and d-TG had partially converted these to 2,4,5-T in 30 min, and completely converted them in 24 hours. When the wheat coleoptile sections were treated with inhibiting doses of l-TG and d-TG these were not hydrolyzed and both compounds appeared on the chromatograms.

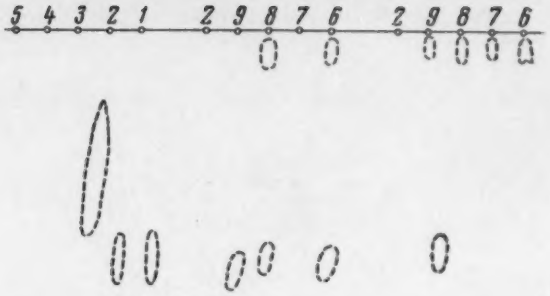

Fig. 1. Chromatograms of wheat coleoptiles treated with stimulating and inhibiting doses of potassium salts. A) stimulating doses of 2,4,5-T and TAB for 24 hr; B) Stimulating doses of l-TG and d-TG for 24 hr; C) inhibiting doses of l-TG and d-TG for 48 hr. Spots of the following were placed on the starting line: 2) 2,4,5-T; 4) TAB; 6) l-TG; 8) d-TG, as well as the substances separated from solutions of the compounds with which the wheat coleoptiles were treated: 1) water; 3,5,7 and 9) potassium salts; 3) 2,4,5-T; 5) TAB; 7) l-TG; 9) d-TG.

Coleoptile sections treated with solutions of TAB did not hydrolyze it at either concentration. Moreover, TAB was apparently absorbed by the coleoptile tissues since it did not appear on the chromatograms. The formation of 2,4,5-T from l-TG and d-TG, and the absence of this compound when the coleoptiles were treated with TAB is evidence that the ability of wheat enzymes to hydrolyze substituted amides of 2,4,5-T does not depend on the optical isomer of the amide group, but on its size, form, and perhaps other physicochemical factors. Apparently the stimulating effect of 2,4,5-T on wheat coleoptiles was inherent, and the activity of l-TG and d-TG depended on their hydrolysis by the plant. The mechanism of the stimulating action of TAB was different and was not associated with its hydrolysis. Inhibiting doses of l-TG and d-TG have a depressing effect on the hydrolytic enzymes of wheat. Apparently the inhibiting effect of 2,4,5-T was also due to the depression of the activity of these enzymes.

It is possible, however, that the hydrolytic reaction is not specific for 2,4,5-T, but represents a secondary phenomenon which is closely associated with the mechanism of 2,4,5-T action, and that various amide groups (in our case fragments of glutamic acid molecules) do not interfere with its action.

If it is found out that other plant species react differently to treatment with amides of 2,4,5-T than wheat then further investigations may reveal a new group of selective herbicides.

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THE EFFECTIVE APPLICATION OF MICRODOSES OF MOLYBDENUM IN COMBINATION WITH GRANULATED SUPERPHOSPHATE TO INCREASE THE PRODUCTIVITY OF PERENNIAL GRASSES

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At the present time the application of small doses of granulated superphosphate added to the seeds before sowing has a very sound physiological basis [1] since superphosphate is one of the most important active links in the system of plant nutrition.

In a paper published earlier from this laboratory [2] it was shown that in the chernozem regions of Kamenny Steppe in the Voronezhsky Province the effectiveness of this method in grass plantings (legume-cereal-grass mixtures) could be increased substantially by enriching the granules of superphosphate with microdoses of boron and molybdenum (300 g ammonium molybdenate and boric acid per hectare portion of superphosphate). By thus enriching the superphosphate applied during sowing with boron and molybdenum, in addition to the increase in grass yield we were also able to produce a considerable change in the composition of the biota of the grass field; in respect to the higher plants there was a predominant development of the leguminous components at the expense of the grasses, and in respect to the microbe composition of the rhizosphere, there was a sharp increase in the establishment of azotobacter and nodule bacteria (judging by the development of nodules). This latter circumstance indicated the possibility of increasing the effectiveness of bacterial enrichment (nitrogen-fixing bacteria and azotobacter) in grass plantings; this was confirmed by data from field experiments [2].

From the practical point of view, all of this collectively disclosed the possibility of being able to achieve a considerable increase in grass yields in areas of inadequate mineral fertilizer by using only very small doses of these minerals.

As we have indicated, our earlier experiments with grasses were done on chernozem soils in a zone of inadequate moisture. The cultivation of perennial grasses during the crop rotation of fields is considerably more effective in the non-chernozem zone where there are bases for systematically getting higher, and especially more stable, grass yields, and where the existence of a high yielding grass field is determined by a high degree of cultivation.

Therefore, it seemed necessary to check the effectiveness of these measures in areas with podzolic soils in the non-chernozem zone. Such experiments were carried out during a four year period (from 1954 until 1957) on the experimental farm "Snigiri", the Division of Biological Sciences, Academy of Sciences USSR, Krasnogorsky Region, Moscow Province.

The soils on the fields of the crop rotation experimental farm were moderate and heavy wood-podzolic and silt loams (examined by N. P. Karpinsky and M.I. Kamyniny in 1948). They were extremely poor in free phosphoric acid (as determined by A.T. Kirsanov's method), and were also in need of lime; the lime requirement in figures was in the order of 6-7 T $CaCO_3$ per hectare (when calculated on the basis of the amount of hydrolytic acids). In the past about 3 T of lime per hectare were applied on all the crop rotation fields for either one or two rotations. In view of the inadequacy of this dose and its depletion by the time our experiments were begun, the soil in all the fields was found to be quite acid when the pH was measured in a KCl solution (from pH 4.3-4.4 in the less cultivated fields, to 5.0-5.2 in the more cultivated ones). In all cases there was also a considerable

quantity of free aluminum, as determined by Sokolov's method. The corresponding data are given in the section dealing with experimental results.

The climatic conditions were quite varied during the four years of the experiments, beginning with an extremely dry year in 1954 and ending with a comparatively cold summer with profuse rains in 1957.

Fig. 1. The effect of molybdenum on the growth of clover. 1 and 2) granulated superphosphate enriched with molybdenum at the rate of 150 g Mo per 50 kg superphosphate added with the seeds; 3 and 4) granulated superphosphate without molybdenum added with the seeds.

Preliminary investigations of the vegetative experiments performed by T.A. Akimochkina showed that of the two microelements, boron and molybdenum, which were used to enrich the superphosphate granules in the experiments on the chernozem soil at Kamenny Steppe only one, molybdenum, had an effect on the growth of clover on podzolic soil at Snigiri; the growth of the plants as well as the accumulation of vegetative mass and their reproductive development was also accelerated (Fig. 1). The addition of boron to the molybdenum or else the enrichment of superphosphate with just boron did not have any positive effect. Because of this we investigated the effect of molybdenum only in the field experiments.

Methods Used in the Field Experiments

The experiments were done in duplicate under field conditions on corresponding crop-rotation fields with the plots measuring about 0.15 hectare. With the object of including all the elements of the microrelief of the given field, and excluding occasional variations, in the results the plots consisted of narrow strips which were three passes of the drill in width (4.5 m), and extended 350-400 m in length.

The experiments were layed out with the same design as the fall crop (oats), with which additional sowings of grasses were made, as well as plantings of grasses themselves, i.e. the same mineral nutrients in the same doses were introduced into the rows when oat seeds were planted and when grass seeds were planted.

The granulated superphosphate was mixed with the seeds immediately before planting at the rate of 50 kg fertilizer (about 10 kg P_2O_5) per hectare portion of seeds.

Molybdenum was applied in the form of ammonium molybdate by two methods. In one of these the necessary amount of salt was dissolved in a small amount of water which was slightly warmed; the solution was used to gradually moisten the granulated superphosphate by sprinkling during careful stirring. Using this method, 300 g of ammonium molybdate were added to a hectare portion of superphosphate (50 kg); this corresponded to

about 150 g molybdenum per hectare. The volume of solution was small (about 2 liters per 50 kg superphosphate). After the treatment the superphosphate was almost dry.

TABLE 1

The Effect of Microdoses of Molybdenum in Combination with Granulated Superphosphate on the Productivity of Legume-Grass Mixtures. (yield of hay in centners/hectare)

Variants	First year of treatment				Second year of treatment				Third year of treatment		Average of all the data for 3 years				
	total mass measurement		measurement on small plots		total mass measurement		measurement on small plots		measurement on small plots*		yield	increase due to fertilizer		increase due to molybdenum	
	yield	increase	yield	increase	yield	increase	yield	increase	yield	increase		incentner/hectare	in % control	incentner/hectare	in % control
Control (without addition of fertilizer)	38.1	-	33.2	-	20.3	-	24.4	-	34.3	-	30.1	-	-	-	-
Granulated superphosphate with the seeds	48.8	10.7	37.3	4.1	24.7	4.4	31.2	6.8	49.7	15.4	38.4	8.3	27.6	-	-
The same + 15 g/hectare Mo by moistening the seeds	54.2	16.1	40.4	7.2	29.0	8.7	36.3	11.9	56.3	22.0	43.2	13.1	43.5	4.8	15.9
Granulated superphosphate, enriched with Mo (150 g/hectare), with the seeds. . .	62.3	24.2	43.8	10.6	30.1	9.8	40.5	16.1	60.5	26.2	47.5	15.8	57.8	9.1	30.2

*We were unable to make the necessary weighings of the total yield this year.

According to another method which made it possible to limit oneself to smaller doses of nutrients, the plants were supplied with molybdenum by treating the seeds themselves with a solution of ammonium molybdate before sowing instead of enriching the superphosphate. It should be noted that a considerable increase in seed moisture makes presowing treatment of seeds with solutions difficult. Therefore we tried to merely apply the material to the seeds without increasing their moisture content substantially. In order to do this we used only 250 ml of solution to treat a hectare portion of seeds.

There were 30 g ammonium bolybdate, or about 15 g Mo, in this volume of liquid, i.e. the dose was 10 times smaller than it was with the granulated superphosphate. It was not expedient to use equal doses of molybdenum in both methods since a dosage of 300 g salt per hectare portion of seeds during careful treatment of the seeds had a harmful effect, and a dosage of 30 g per hectare when applied to the superphosphate was extremely inadequate.

In the variants inoculated with bacteria the grass seeds were treated with preparations of azotobacter and nitrogen-fixing bacteria. The seeds were mixed with the mineral nutrients and inoculated about an hour before sowing. The seeds were sown with a ten-row disc drill as follows; the oat seeds were sown first to a depth of 3-4 cm, then the grass seeds to a depth of 1-2 cm.

In most cases the grass yield was calculated by two methods: 1) by cutting a strip 22.5 m wide across all the small plots with a sickle in both replicates in 4 to 5 evenly spaced places along the entire length of the strip which was, as we described, 300-400 m long. After the amount of hay in the sample sheaves was determined,

the yield of grass which was calculated for each variant on the basis of the weight of the green mass was converted to yield of hay. 2) by a measurement of the total mass on the remaining area of the plot. The grasses were cut with a horse-drawn mower, and the yield of hay was based on weight of the wagon loads.

The results pertaining to yield obtained by each method of calculation are given below separately.

The present report contains only results of experiments with grasses. Results of experiments with oats, as well as with winter wheat (wheat- couch grass hybrid), will be reported in a separate paper.

Results of Experiments with Perennial Grasses

The first experiment with grasses was started in 1954 in field No. 1 of crop rotation, the soil was cultivated to an average degree (pH in KCl 4.7-4.8; mobile aluminum 3.0 mg per 100 g). In 1946 lime was applied to this field at the rate of 3T/hectare. The grass mixture, consisting of red clover, alfalfa, orchard grass, fescue, and timothy was planted in the spring of 1954 with the oats; this had been preceded in 1953 by potato fertilized with manure at the rate of 20 T/hectare. In this experiment grass continued in the same field for three years, from 1955 until 1957, making it possible to evaluate the duration of the effect of the mineral nutrients applied in 1954 when the grass seeds were planted.

The grass yield data for each year, as well as the average of three years, is given in Table 1.

Regardless of the considerable variation in the absolute value of the yield obtained by both methods of calculation, it is remarkable that in spite of the extreme length of the plots there was such a regularity concerning the effect of the fertilizer applied during the three years for the large (average data of two measurements) as well as the small (average data of 8-10 measurements) plots. This imparts a high degree of validity to the conclusions.

The data in Table 1 indicate the prolonged effect of the fertilizer applied once with the seeds in 1954; it did not decline (but even increased) even during the third year of treatment (i.e. during the fourth year of plant growth). Granulated superphosphate alone applied in a small dose with the seeds ($\frac{1}{2}$ centner/hectare) resulted in a considerable increase (27% of control). This increase more than doubled when 150 g molybdenum per hectare were added with the superphosphate. Even a dose of 15 g molybdenum per hectare produced quite a substantial effect on the yield of hay, even though the effect doubled with the application of 150 g.

TABLE 2

The Effect of Microdoses of Molybdenum in Combination with Granulated Superphosphate on the Ratio of Grass Components in the Yield of Hay (First Year of Treatment)
(in % dry weight of hay)

Variants	Legumes			Grasses					Variety of grasses (weeds)
	clover	alfalfa	total	orchard grass	fescue	timothy	grains	total grasses	
Control	10.8	1.3	12.1	2.2	26.9	13.2	21.1	63.4	24.5
Granulated superphosphate with seeds	19.9	2.4	22.3	4.0	28.1	10.9	19.0	62.0	15.7
The same + 15 g/hectare Mo by moistening the seeds	33.2	2.3	35.5	4.5	28.0	6.1	14.1	52.7	11.8
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	36.4	1.8	38.2	1.3	24.4	6.4	14.3	46.4	15.4

An analysis of the plant composition of the hay revealed that both superphosphate and molybdenum changed the interspecies ratio in the grass community in the same direction: as a consequence the growth of the leguminous components, especially clover, increased as well as their ratio in the stand of grass due to the decrease in the abundance of grass components, especially the weeds.

During the second year of grass treatment the ratio of weeds in the stand of grass was reduced almost to nothing. However, the predominance of legumes over the grasses in the composition of the hay due to the influence of granulated superphosphate, and especially due to the superphosphate enriched with molybdenum, was also present during the second year of treatment (Table 3).

TABLE 3

The Effect of Microdoses of Molybdenum in Combination with Granulated Superphosphate on the Ratio of Components in the Yield of Hay (Second Year of Treatment) (in % dry weight of hay)

Variants	Legumes	Grasses	Weeds
Control	46.5	51.9	1.6
Granulated superphosphate with seeds	64.5	35.1	0.4
Granulated superphosphate enriched with Mo (150 g/hectare) with seeds	73.5	26.1	0.4

It is also quite significant that due to the effect of superphosphate, and to even a greater degree superphosphate enriched with molybdenum, the number or roots and their nitrogen content increased. The results of a calculation of the number or roots in different layers of the soil at the end of the second year of treatment (fall of 1956) together with further determinations of their nitrogen content (Kjeldahl) are given in Table 4 (see also Fig. 2).

TABLE 4

The Change in Weight of the Roots (in centner/hectare) and Their Nitrogen Content as the Result of Treatment with Granulated Superphosphate and Molybdenum Introduced with the Seeds during Sowing (at the End of the Second Year of Treatment) (the roots were washed in layers from a block measuring 33 x 30 cm)

Depth of the soil layer (in cm) from which the roots and indicators of the experiment were washed	Without fertilizer	With granulated superphosphate	With granulated superphosphate enriched with molybdenum (150 g/hectare)
0- 20	45.9	93.9	133.3
20 - 40	2.9	4.0	8.4
40 - 60	1.3	1.6	1.9
Total dry weight of roots	50.1	99.5	143.6
Nitrogen content of roots, in % dry weight	1.2	1.3	1.3
Total nitrogen content, in kg/hectare	58.2	128.3	182.4

As we can see from Table 4, even when only granulated superphosphate was introduced with the seeds, the weight of the roots of the grasses increased twofold by the end of the second year of treatment; when superphosphate was enriched with molybdenum the weight increased almost threefold. The nitrogen content of this material increased to a comparable degree; in grasses fertilized with superphosphate enriched with molybdenum it rose to 182 kg/hectare in place of 58 kg/hectare in the control plants. The significance of these figures in the evaluation of the mechanical role of grasses and their role in improving the nitrogen balance in agriculture hardly needs explanation.

The experiment described was performed, as we have said, on soil which was cultivated to a moderate degree. The second experiment started in 1956 on field No. 3 of a soil rotation scheme was even more effective; this soil received less fertilizer previously and was also characterized by a lower degree of cultivation. The soil acidity was considerably higher than in field No. 1 (pH in KCl – 4.3-4.4). The content of mobile aluminum was also higher (4.0 mg per 100 g soil). This experiment was performed in the same way as the previous one, but with two variables – with and without inoculation of the seeds with preparations of nitrogen-fixing bacteria and azotobacter. Oats, with which the grasses were sown, followed a vetch- oats mixture for seed. In this case the grass mixture consisted of clover and timothy.

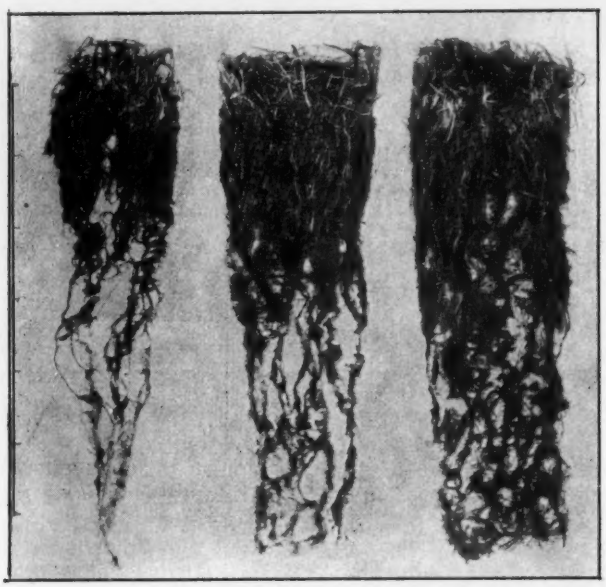

Fig. 2. The effect of granulated superphosphate and molybdenum introduced with the seeds during sowing on the development of the root system in perennial grasses. Roots washed from a block measuring 30 x 33 x 60 cm. 1) control; 2) granulated superphosphate with seeds; 3) granulated superphosphate enriched with molybdenum added with the seeds.

In this experiment the effect of the addition of fertilizer on the grasses was expressed very clearly both in respect to the density of the grass stand and in respect to the vigor of plant growth (especially clover) and the intensity of the green coloration.

The data pertaining to the harvest are given in Table 5; from these it is evident that in this experiment the results of measurements on the small plots agree even more closely with data from the total mass measurements than they did in the experiment described above. Furthermore, the nature of the effect was the same when the grass seeds were sown after inoculation, or when they were sown without inoculation with bacteria.

From Table 5 it is evident that the effect of granulated superphosphate both in respect to the absolute increase in yield of hay (about 8 centner/hectare) and in respect to the comparative increase (25-28% of control) corresponds approximately to an average effect which was also obtained in the experiment described previously. The effect of molybdenum appeared to be considerably greater here. Enriching the granulated superphosphate with 150 g molybdenum per hectare resulted in an increase of its effectiveness by three to four times. Even a dose of molybdenum consisting of 15 g/hectare more than doubled the increase in yield of hay, as compared with the increase due to superphosphate alone (without molybdenum). And finally, from the figures in Table 5 it is evident that the addition of molybdenum had a determining effect on the effectiveness of the

bacterial preparations. Without molybdenum the effect of these preparations lay within the limits of the accuracy of the experiment (increase of 2.4-4.7%); with molybdenum the increase in yield of hay reached 4-5 centner/hectare, or 13-16% of the control. Accompanying the joint action of granulated superphosphate enriched with molybdenum, and the inoculation with bacterial preparations, the yield of hay doubled; molybdenum played a leading role in this total effect. Superphosphate played a secondary role, and bacterial inoculation a third.

TABLE 5

The Effect of Microdoses of Molybdenum in Combination with Granulated Superphosphate and Inoculation with Bacterial Preparations on the Productivity of Legume-Cereal Grass Mixtures (yield of hay, in centner/hectare during the first year)

Variants of experiment	Total mass measurement		Measurement on small plots		Average of all indicators				
	yield	increase	yield	increase	yield	increase due to addition of mineral fertilizer		increase due to bacterial inoculation	
						in %	ctn/ha	in %	ctn/ha
Control (without fertilizer)	28.7	—	32.3	—	30.5	—	—	—	—
Granulated superphosphate, with weeds	37.7	9.0	40.3	8.0	39.0	8.5	27.9	—	—
The same + 15 g/hectare Mo via soaking of seeds	44.8	16.1	52.9	20.6	48.9	18.4	60.3	—	—
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	53.4	24.7	60.9	28.6	57.2	26.7	87.6	—	—
Azotobacter and nigrogen-fixing bacteria	30.5	1.8	33.3	1.0	31.9	—	—	1.4	4.6
The same + granulated superphosphate, with seeds	39.6	10.9	39.9	7.6	39.8	7.9	24.7	0.8	2.7
The same + granulated superphosphate + 15 g/hectare Mo via soaking of seeds	50.5	21.8	55.1	22.8	52.8	20.9	65.5	3.9	12.8
The same + granulated superphosphate enriched with Mo (150 g/hectare) with seeds	59.4	30.7	64.7	32.4	62.1	30.2	94.7	4.8	15.7

In addition to the effect of small doses of granulated superphosphate and microdoses of molybdenum introduced during the sowing of grass seeds on the yield of hay, a corresponding effect on the nutrient quality of the grasses also appeared.

The results of analyses on the content of total nitrogen (according to Kjeldahl) and protein nitrogen (Barnstein) in clover and timothy from the experiment just described are given in Table 6. From the data in Table 6 there immerged the somewhat suprising and, up until now, difficult to explain fact of the clearly expressed decrease in the use of nitrogen for the synthesis of protein by the plants as the result of the influence of granulated superphosphate introduced with the seeds. This was observed in clover and timothy, in plants of the first harvest as well as those of the second harvest. This phenomenon is even of greater interest since it was observed when the plants were extremely difficient in phosphorus, and their yield was increased significantly as the result of applying granulated superphosphate with the seeds (see Table 5).

The addition of molybdenum to the superphosphate via the roots changed the picture. As a result, not only was the unfavorable effect of superphosphate on the synthesis of protein removed, but in many cases, expecially clover, the degree to which nitrogen was used for the synthesis of protein in the fertilized plants even markedly exceeded the degree of its utilization in the control plants. Since the total nitrogen content of clover

also increased greatly as the result of the application of superphosphate enriched with molybdenum, as a result the percentage content of protein nitrogen in the leaves and stems of this plant increased one and one-half to two times. The significance of this in directing not only the yield of grasses, but also their nutrient value can hardly be overestimated.

TABLE 6

The Effect of Granulated Superphosphate and Microdoses of Molybdenum on Total Nitrogen and Protein Nitrogen Content of Grasses (in % dry weight)

Variants of experiment	Plant from first harvest			Plants from second harvest		
	total nitrogen	protein nitrogen	protein nitrogen in % of total	total nitrogen	protein nitrogen	protein nitrogen in % of total
Clover (leaves)						
Control	2.91	2.44	84	3.78	3.13	83
Granulated superphosphate, with seeds	3.22	2.48	77	3.88	2.90	75
The same + 15 g/hectare Mo via soaking of the seeds	3.66	3.04	83	4.58	3.78	83
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	4.20	3.63	87	4.77	4.29	90
Clover (stems)						
Control	1.30	0.89	68	1.34	1.07	80
Granulated superphosphate, with seeds	1.68	0.95	57	1.26	0.96	76
The same + 15 g/hectare Mo via soaking of the seeds	1.86	1.42	76	1.65	1.50	91
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	2.42	2.07	86	1.79	1.50	84
Timothy (entire plant)						
Control	1.44	1.14	79	2.43	2.09	86
Granulated superphosphate, with seeds	1.51	0.92	61	1.88	1.45	77
The same + 15 g/hectare Mo via soaking of the seeds	1.51	1.21	80	2.24	1.98	88
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	1.58	1.26	80	2.31	2.02	88

In addition to the effect on the synthesis of protein and the total nitrogen content of the plants superphosphate as well as molybdenum, especially, brought about a significant change in chlorophyll and carotene content of the grass leaves; this also was of substantial significance in the nature of their nutrient value.

It is evident from Table 7 that these changes were expressed most clearly in clover where superphosphate enriched with moblydenum induced a twofold increase in carotene content (determined by the method used at the All-Union Vitamin Institute), and a two and one-half times increase in chlorophyll content. The changes in timothy progressed in the same direction, but were not expressed as clearly. It is interesting to note that the increase in chlorophyll content of clover was distributed more or less evenly between chlorophyll a and b (determined according to T.N. Godnev), whereas in the meantime, in timothy the increase was primarily in chlorophyll a, and only a small degree in chlorophyll b.

In Table 7 note the extremely sharp increase in chlorophyll content of clover leaves following an increase in the dosage of molybdenum from 15 to 150 g/hectare. This also corresponded with the external appearance of the plants which were particularly dark green at high doses of molybdenum.

Once supplied with the total results of the experiments with grasses, one has a sufficient basis to conclude that the application of micro doses of molybdenum in combination with a small dose of granulated superphosphate makes it possible to obtain an increase in the production of roots and a considerable improvement in the nutrient

qualities of perennial grasses on podzolic soils with only a small application of fertilizer. It also opens up the possibility of a more effective use of bacterial inoculations (nitrogen-fixing bacteria and azotobacter) with these grasses.

TABLE 7

The Effect of Microdoses of Molybdenum in Combination with Granulated Superphosphate on the Chlorophyll and Carotene Content of Fresh Clover and Timothy Leaves

Variants of experiment	Chlorophyll in %			carotene in mg %
	a	b	total	
Clover				
Control	1.30	0.46	1.76	9.05
Granulated superphosphate, with seeds	1.56	0.59	2.15	13.88
The same + 15 g/hectare Mo via soaking the seeds	1.88	0.69	2.57	15.97
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	3.14	1.11	4.25	18.27
Timothy				
Control	1.98	0.81	2.79	—
Granulated superphosphate, with weeds	2.33	0.86	3.19	7.69
The same + 15 g/hectare Mo via soaking the seeds	2.36	0.88	3.24	—
Granulated superphosphate enriched with Mo (150 g/hectare), with seeds	2.58	0.98	3.56	9.92

The next problem is an extensive production investigation of this method on other soil types, especially in the regions of the non-chernozem zone.

SUMMARY

The present work discusses a further development of earlier published investigations. It was shown that the productivity of perennial leguminous-cereal plant mixtures could be greatly enhanced by enriching granulated superphosphate with microdoses of molybdenum (about 150 g. per hectare). The enriched fertilizer was introduced into the soil simultaneously with the seeds.

These microdoses of molybdenum and superphosphate introduced into the soil with the seeds continued to exert a highly effective influence even in the third year of treatment (fourth year of life) of the grasses.

The effect of the enriched fertilizer was double that of superphosphate without molybdenum, and in soils of high acidity and high content of mobile aluminum the effect increased as much as 3 to 4 times.

The increase of crop yield was mainly due to better growth of the leguminous components of the plant mixture (clover and lucerne), whereas the role of the cereals and especially of weeds was smaller.

Granulated superphosphate enriched with molybdenum and introduced into the soil with seeds also profoundly influenced the bacterial preparations (nitrogen-fixing and azotobacter) applied at sowing. In experiments on soils of high acidity innoculation of seeds with the aforementioned preparations in the absence of molybdenum did not exert any influence on the crop yield of hay. Inoculation of seeds in conjunction with molybdenum – enriched superphosphate resulted in a noticeable increase of the crop yield.

Besides enhancing the crop yield, molybdenum also enhanced the nutritive value of the leguminous and cereal components of the grass mixture: nitrogen absorbed by the plant was more fully used for synthesis of

albumin, the content of carotene and chlorophyll in the plant greatly increased, and the total amount of nitrogen increased in clover. Under conditions of molybdenum deficiency granulated superphosphate introduced simultaneously with the seeds at sowing had a negative influence on the utilization of stored nitrogen and on the synthesis of albumin. This negative influence was removed by molybdenum.

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METHODS

SEPARATION OF AMINO ACIDS ON SMALL DIMENSION PAPER CHROMATOGRAMS

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The method described below for separating amino acids on paper chromatograms of small dimensions is designed primarily for phytophysiological investigations, in which it is very frequently necessary to perform a series of determinations with several variants. In such cases the use of the usual large chromatograms is time-consuming and very impractical because of the cumbersome chambers, and the great consumption of chromatographic paper, solvents and reagents. Taking this into account the authors of the present paper tried several means to decrease the dimensions of the chromatograms to a point at which the separating ability still remained the same as it had been in the large chromatograms.

It should be noted that although the literature contains a description of methods for separating amino acids on chromatograms [1, 2] of small dimensions, these methods are limited since the separating capacity* of these chromatograms is small and only 5-7 amino acids can be separated.

We were able to increase the separating capacity of the chromatograms by several means, and because of this we were able to decrease their dimensions almost by half (to 12-20 cm), as compared with the large chromatograms usually used which are 40-60 cm in length. The method which we worked out was used for two years at the Institute of Plant Physiology, AN SSSR, as well as at various other institutes in the USSR. The present paper contains a brief description of this method.

Paper. In order to get a chromatographic separation of amino acids by the method described, either slow or regular (not very rapid) paper from the Volodarskii Leningrad Factory No. 2 can be used. Especially good results were obtained with experimental paper from the Goznak Leningrad Factory.

Impregnation of the paper with a buffer. Large sheets of chromatographic paper were cut into small sheets measuring 16 x 25 cm. These small sheets were immersed individually for 5-10 sec. into a container with a 1/15 M solution of phosphate-citrate buffer of pH 4 or pH 6. From here they were transferred to a clean glass, and the excess liquid was removed with filter paper; following this the sheets were suspended and dried at room temperature.

Form of chromatograms. All the known forms of chromatograms [3-5] can be used in this method: round, radial sections, wedge-shaped, or semi-wedge-shaped, and also one-and two-dimensional rectangles.

All of these types have a different separating capacity; it is greatest in the round chromatograms and least in the rectangular ones; radial sections and semi-wedge-shaped chromatograms occupy and intermediate position in this respect [6].

Although the choice of the form of the chromatogram frequently depends on circumstances and the purpose of the chromatographic separation, however, the semi-wedge-shaped form shown in Fig. 1 is the one used more universally.

*The separating capacity of the chromatograms is here understood to mean the ratio of the size of the secondary spots along the direction of the movement of the solvent to the interval between the beginning and the center of the maximum density of the secondary spots.

Application of primary spots at the start of the chromatogram. The primary spots are applied at the start of the chromatogram with a micropipette graduated in 0.1-0.2 ml, or else with a slender capillary. It is very convenient to apply them by means of a platinum loop with the diameter of the loop being about 1 mm. In order that the loop always take up the same volume of liquid it should be immersed into the solution only up to the depth of the loop. It is essential that the size of the primary spots should be as small as possible and not exceed 3 mm.

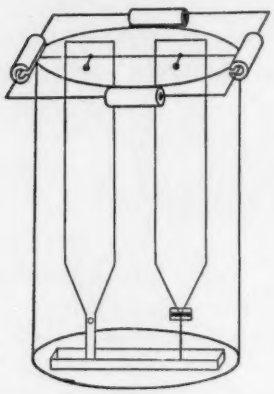

Fig. 1. Chromatographic chamber with two semi-wedge-shaped small dimension chromatograms (15-20 cm long; 3 cm wide). Left chromatogram with a paper wick on which one can see the small circle of the primary spot (start); length of the wick 2-3 cm, width 0.4 cm. Right chromatogram with a thread wick, fastened on with a glass clamp; the construction is clarified in Fig. 2B. The primary spot was placed on the upper part of the thread. See the text for description of the chamber.

If the concentration of amino acids in the experimental solution is low, and the chromatograms obtained are pale, the solution should be either evaporated or applied on the primary spot several times. In the latter case, before each successive application the spot should be completely dry, otherwise its dimensions will increase.

In place of the tedious repeated application of a weakly concentrated solution to the chromatogram, the following method can be used. Cut semi-wedge-shaped strips about 10 cm long and 3 cm wide from chromatographic paper, or even better, from round nonash filter discs (Fig. 2A). Fasten the base of each strip between two glass rods held together with rubber bands, after this with a micropipette place 0.2 ml of the experimental solution at once on the strip, so that the lower part of about 1 cm remains unmoistened. After the strip is dry, in the same manner apply the following portions of solution on the strip for as long as necessary. Following this, roll the strip lengthwise into a tight tube, then place this into a shorter glass tube (about 5 m long and about 3-4 mm in diameter). In addition, using a glass clamp (construction of which is clarified in Fig. 2B), attach a heavy thread to the protruding sharp end of the rolled strip. White thread "Iris" No. 10 serves very well for this purpose after it has first been boiled in three changes of distilled water.

The rolled strips thus arranged are then suspended in a glass chamber; their lower end is immersed in a groove with 50% alcohol, and the ends of the threads are fastened outside of the chamber between two horizontal glass rods (Fig. 2 C). The top of the chamber is covered with two glass plates in such a way that a crack 4-6 mm wide remains between them, the threads pass out through this crack without touching the edges of the glass slides. The 50% alcohol, which rises along the rolled strip by capillary action, dissolves the amino acids and other substances and transfers them to the thread. In the region of the crack the solvent evaporates, and the dissolved substances precipitate and accumulate on the thread thus forming a slightly colored part which corresponds to the initial spot. Several hours are required before the amino acids are completely transferred from the strips to the threads, therefore it is best to let the entire arrangement stand overnight.

After the transfer the threads are separated from the strips, dried, and trimmed to 0.5 cm from the initial spot at the top, and at a distance of 2-3 cm from it at the bottom so that the entire section is 4-5 cm long. Using a glass clamp, the upper end of this section is then fastened to the semi-wedge-shaped chromatogram which has no paper wick (Fig. 1, right).

When the lipoid content of the substances is high, after the extract has been applied, dried, and the strip rolled up, it is recommended that the strip be rinsed first with ether or benzene using the same procedure described.

Chromatographic chambers. Glass chambers which are either round or rectangular in cross section and 15-20 cm high can be used as chromatographic chambers (culture dishes, battery jars, and aquaria). The number of chromatograms which can be placed in the chamber at one time depends on the diameter of the chamber. A narrow groove containing the solvent is placed at the bottom of the chamber. The chamber is covered on the top with two glass slides which are equipped with four sections of heavy rubber tubing (vacuum),

cut length wise to the cavity, to prevent slipping (Fig. 1). In order that the chromatograms should not slip down, each one of them is fastened immediately above the glass with a pin; after this both slides are pushed more closely together.

One of the special features of the described procedure (see Fig. 1) is the fact that the solvent rises along the wick and chromatogram, and eventually passes to the extruding end; here it evaporates, and in this way forms a continuously rising stream along the chromatogram. This principle has been put to use previously with the circular chromatograms [7 - 9]. The chamber should be placed in an exhaust hood or in a well-ventilated place.

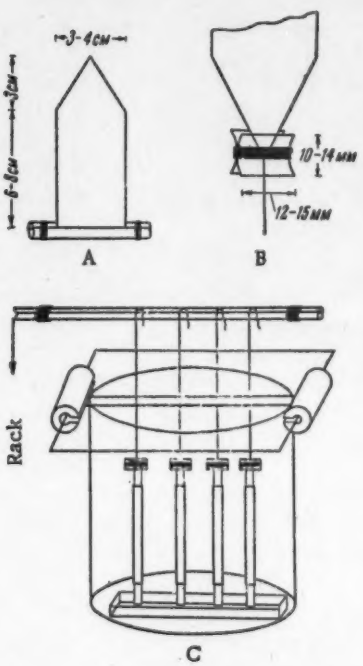

Fig. 2. A device for applying many spots of low concentrated solutions (see text). A) Semi-wedge-shaped strip; B) glass clamp for fastening the thread to the semi-wedge-shaped strip, or to the chromatogram; the bent halves of the clamp are cut from a glass ring 3-4 cm in diameter, and held together with a rubber band (shown in black); C) arrangement for transferring the amino acids from the semi-wedge shaped strip to the thread (see text).

Suitable solvents and the sequence of amino acids distribution on chromatograms. After separation is complete the top layers of the following mixtures are used for the chromatographic separation of amino-acids:

A. Butyl alcohol – conc. acetic acid – 1/15 M phosphate-citrate buffer pH 4, in a volume ratio of 4:1:5;
B. Butyl alcohol – conc. acetic acid – 1/15 M phosphate-citrate buffer pH 6, in a volume ratio of 4:1:5;
C. Butyl alcohol – conc. formic acid – 1/15 M phosphate-citrate buffer pH 4, in a volume ratio of 4:1:5.

The paper should be saturated with a buffer of the same pH as used in the mixture (see above).

The solvent mixtures given here differ from those usually used in that a phosphate-citrate buffer replaces the water. According to a verbal report by O.P. Osipova, such an exchange accompanied by a saturation of the paper with the same buffer improves the quality of the chromatogram. This was also confirmed in our special experiments. If butanol saturated only with a phosphate-citrate buffer is used for a chromatographic separation, as McFarren [10] has done, the separation of amino acids proceeds three-four times slower than it does when acetic acid or formic acid is present; we observed no difference in the quality of the chromatograms in either case. It should also be noted that the specific pH in this case is conditional since its specific value in the butanol layer during chromatographic separation is difficult to determine because of the nonaqueous medium, the effect of the charge on the paper, and the variation in the solubility coefficients of phosphate and citric acid in a butanol-water system.

The choice of solvent mixtures (A, B, C) for paper chromatography depends on the combination of amino acids in the solution being investigated. Mixture A is a better solvent for the tryptophane-methionine-valine group, and asparagine separates from arginine better in this mixture. Lysine separates better from histidine in mixture B, as well as aspartic acid from the glycine-serine-glutamine group. The distribution of the amino acids on the chromatograms is the same in both of these mixtures. Mixture C gives a different distribution of some of the amino acids and is used primarily for separating the aspartic acid-glutamine-serine-lysine groups. Just as on the large chromatograms, these solvent mixtures do not completely separate some of the amino acids. When a mixture of A and B is used the following separate one over the other: glutamine, serine and glycine; hydroxyproline and glutamic acid; tyrosine and α-amino butyric acid. When mixture C is used the following pairs of acids coincide: lysine and hystidine; glycine and hydroxyproline; glutamic acid and threonine; alanine and proline; tyrosine and α-amino butyric acid; methionine and trypotphane. The separation of superposed amino acids by developing the chromatograms with isatin is described below.

The distribution of the most abundant amino acids in solvents A, B and C is given in a table below.

Duration of chromatographic separation. The time required for the separation of the acids depends greatly on the density of the chromatographic paper, length and width of the wick, and the temperature. Furthermore, the translocation rate of the individual amino acids along the chromatogram varies so much that they can ultimately easily be separated in this respect into two groups: the rapid and the slow moving ones; either proline or alanine (see table) is taken as the boundary between these two groups. Depending on the conditions 20 to 40 hours are necessary for the separation of the slowly moving amino acids; this time is too long for the rapidly moving amino acids, they overlap at the evaporating margin of the solvents; therefore they are separated on another chromatogram during the elapse of 7-10 hours.

TABLE

The Distribution of Amino Acid Spots on Chromatograms Using a Mixture of Solvents A, B and C, and the Staining of the Spots When the Chromatograms Were Developed with Ninhydrin and Isatin Accompanied by an Adequate Treatment with Magnesium Sulfate (Method I) and Sodium Silicate (Method II)

Abbreviations indicating color: br - brown; yel - yellow; gr - green; cin - cinnamon; r - red; ros - rose; gy - gray; bl - blue; vio - violet.

Name of amino acids	Order of distribution		Staining of the spots during development		
	Mixtures A, B	Mixture C	Ninhydrin	Isatin, method I	Isatin, method II
Glutathione	1	1	vio	r	vio-r
Cystine	2	2	cin	from gy to gy-r	gy-vio-r
Lysine	3	3	vio	from vio-bl to gr	from r to vio-r
Histidine	4	4	gy-vio	from gy-ros to gy-gr	from gy-vio to gy-gr
Arginine	5	5	vio	from r to vio-r	vio-r
Asparagine	6	6	cin-vio	from r to vio-r	from vio to bl
Aspartic acid	7	7	bl-vio	from vio to bl-vio	from vio to bl-vio
Glutamine	8	8	vio	r	r-vio
Serine	9	9	vio	from cin-r to r	r-cin
Lysine	10	10	r-vio	r	r
Hydroxyproline	11	11	yel-cin	bl	bl, fading
Glutamic acid	12	12	vio	from r to vio-r	from r-vio to vio
Threonine	13	13	vio	from r to vio-r	yel-cin
α-Alanine	14	14	vio	from vio to vio-bl	from vio to vio-bl
Proline	15	15	yel	bl	bl, fading
α-amino butyric acid	16	16	vio	from r-vio to vio-bl	from vio to vio-bl
Tyrosine	17	17	gy-vio	from gy-vio to gy-gr	from gy-bl to gy-gr
Tryptophane	18	18	gy-vio	from gy-ros to br-gr	from gy-vio to bl-vio
Methonine	19	19	vio	from vio-r to vio	from vio to bl-vio
Valine	20	20	vio	r	r
Phenylalanine	21	21	gy-vio	from r-vio to bl	from vio to bl
Leucine	22	22	vio	from r to vio-r	from r to vio-r

Development of the chromatograms. After the correct exposure in the chamber, the chromatograms are dried at room temperature for 1-2 hours and are then developed. Isatin which gives very characteristic spot colors of the various amino acids is used for developing rather than ninhydrin which stains the spots of most of the amino acids with an identical violet color; this simplifies their identification, as well as their separation in those cases when the adjoining amino acids overlap one over the other. The development of the chromatograms using isatin accompanied by supplementary treatment with magnesium sulfate (method I) was described by us in detail in other papers [11, 12], and therefore at the present we are limiting ourselves to the presentation of data concerning the staining of amino acid spots at various methods of development (see Table).

It is evident from the table that the isatin methods I or II used for developing certain amino acids (for example, lysine, asparagine, threonine) give distinct contrasting colors which can be used as a supplementary index for identifying these amino acids. To do this the chromatogram is cut in half lengthwise, and each half is developed by one of these methods. In this way alanine and proline, which are superposed when mixture B is used, can be separated since the blue color of the latter fades away when developed by method II, while the violet color of alanine is completely retained; the same is true of glycine and hydroxyproline.

The identification of the amino acids on the chromatograms in the method described is based primarily on the position and color of the secondary spots (see Table). When this method is used, and especially in doubtful cases, it is advisable to set up a parallel chromatogram with samples.

When analyzing chromatograms of plant material it is necessary to consider the following. At the present time more than 70 compounds have been found in the extracts and juices of plants which develop with ninhydrin and represent amino acids and various derivatives [13]. Furthermore, about the same number of compounds have been disclosed in various plants which also develop with ninhydrin, but which have not yet been identified. Therefore, even though in most instances "common" amino acids usually appear on plant chromatograms, however, quite frequently there are also spots on them which can not be definitely identified on the basis of position and color.

Because of the absence of corresponding samples or very specific reactions which might facilitate the identification of such spots, it is necessary to confine ourselves to representing them with letters and describing their colors and position on the chromatogram. In order to obtain an accurate identification of the unknown substances one chromatogram is not adequate; it is necessary to isolate at least several milligrams and use various chemical or physical methods of identification. It is expedient to undertake such a special investigation in plant physiology only when there is an adequate basis to assume that the unknown substance plays a highly significant role in the physiological processes studied.

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* See English translation.

REVIEW

MANUAL OF PLANT PHYSIOLOGY

Acad. E. Pop, Prof. N. Salageanu, m. c. al Academiei R. P. R.,
Prof. S. Peterfi, m. c. al Academiei R. P. R., Prof. H. Chirilei

Volume 1, 373 pp. Bucharest, 1957

Plant physiology, which emerged as an independent discipline in 1907 in Roumania with the organization of the first faculty by Emanell Teodoreskii has few textbooks. Several botany textbooks with sections on physiology have appeared in addition to the textbook published by H. Moisesku (1907). In 1951 a Roumanian translation of N.A. Maximov's unsurpassed text appeared but its circulation was inadequate by 5000 copies. In 1957 a textbook by professors H. Chirilei, M. Pushkash and I. Berbat appeared for agricultural and forestry educational institutions.

The appearance of a textbook written by Acad. Emil Pop (V. Babesh University in Kluzh), Prof. Nikolae Salageanu (K.I. Parkhon University, Bucharest) Prof. Stefan Peterfi (Ia. Bol'iai, Hungarian University, Kluzh), and Prof. Haralambie Chirilei (N. Belchesk Agronomy Institute, Bucharest) represents the first large scale attempt to produce a university type textbook for this discipline.

The textbook was designed in two volumes. The recently published first volume contained an introduction and chapters on the following: history of plant physiology, mineral and nitrogen nutrition of plants, photosynthesis, translocation of assimilates, energetics. Each of these chapters was written by a different author.

The chapter "History of Plant Physiology" was written by E. Pop, a well known student of botanical history, and contains an extensive account of the history of physiology and its subsequent development to the present time as an independent discipline. Russian and Soviet investigators, with several exceptions, have played a significant role in the history of this discipline. The section which gives an account of the history of plant physiology in Roumania is new and original; the work of the following is described: E. Teodoresku (physiology and classification of the lower plants, vines, the effect of external conditions on the structure and development of plants), N. Delianu (respiration and biochemistry of plants), S. Ionesku (origin and role of pigments), P. Staneskii (photosynthesis), I. Mikheilesku (leaf physiology), E. Pop (protoplasmic movement, plant ecology), N. Salageanu (photosynthesis, stages of plant development, water relations), and others. This section would have profited considerably if it had been supplemented by data from the research of scientific experimental institutes (Agronomy, Scientific-Experimental Institute, Tobacco Institute) and agronomy and forestry scholars (M. Kiritsesku – Arva – concerning water relations, A. Piesku – concerning the physiology of tobacco and stages of plant development, K. Dzheordzhesku – concerning the physiology of woody plants, G. Valutsa – concerning the developmental stages of agricultural plants).

The next chapter, written by H. Chirilei contains a detailed account of the classical works and present concepts concerning nitrogen and mineral nutrition of plants.

Experiments with labelled atoms are described under the change in methods used for investigating mineral nutrition. However, such important methods as sand, water and soil culture, and also methods of sterile culture, the use of leaf diagnosis, and hydroponics are not mentioned; this represents a substantial shortcoming.

The material presented is combined well with a series of agronomical problems, even though the account relating to the use of fertilizer is too general. It should be noted that this problem was well clarified in the voluminous textbook by Prof. D. Davidesku "Agrochimia", also published in 1957.

The chapter "Photosynthesis", written by N. Salageanu, contains an extensive and complete historical account of the development of experiments concerned with carbon dioxide assimilation, ecology, and the biochemistry of photosynthesis. The author's own investigations concerning the compensation point between photosynthesis and respiration, and photosynthesis in water plants (1939-1949) are cited. N. Salageanu's experiments, in which an original method was used, showed that photosynthesis can occur in very weak light. These data defined more accurately the investigations also performed by A.S. Famintsyn, S.P. Kostychev and V.N. Liubimenko. The graphs and tables, including several original ones, illustrate the text of this chapter well.

The short chapter "Translocation of Assimilates", written by S. Peterfi, contains a brief account of the principal publications; included among these are those by E. Teodoresku and K. Popesku (1915), and the recent investigations by A.L. Kursanov and M.V. Turkin. It would have been appropriate to place this chapter, supplemented with data contained in the chapter on mineral nutrition (pp. 93-97), after the chapter on water relations in plants.

The last chapter, also written by S. Peterfi, is devoted to the problem of energetics; this is examined in detail according to present data concerned with the biochemistry of the respiratory processes, fermentation, and transformation of energy in plants. New data and concepts of Kh. Krebs, O. Warburg, D.M. Mikhlin, M.B. Fedorov and others are discussed. The discussion of information relating to the effect of external conditions on respiration is too abstract.

The account of a succession of data in the literature concerning energy transformation, and the liberation of electricity and light by plants is, in our opinion, well done.

The text is written for scholars, but at the same time it is written in an easily understandable language, even though the same style is not maintained throughout. If a greater uniformity had been maintained in the content of the chapters the text would be more complete. There is a bibiography of the more important papers at the end of each chapter; papers by Roumanian scientists are well represented, even though the latter are referred to very little in the text.

The book is technically satisfactory, but some of the figures are insignificant (Nos. 110, 111, 114), since some of them are of an unsuitable scale. The textbook also contains many misprints.

The publication of a book by four well known Roumanian physiologists is a significant advance in the scientific literature of the region. This will certainly provide the Roumanian biologists and agronomists with up-to-date information concerning the physiology of plants, and will promote the theoretical development and practical use of this information.

Considerably more numbers of the second volume should be published than there were of the first in order to better provide for the ever increasing interest in this discipline in Socialistic Roumania.

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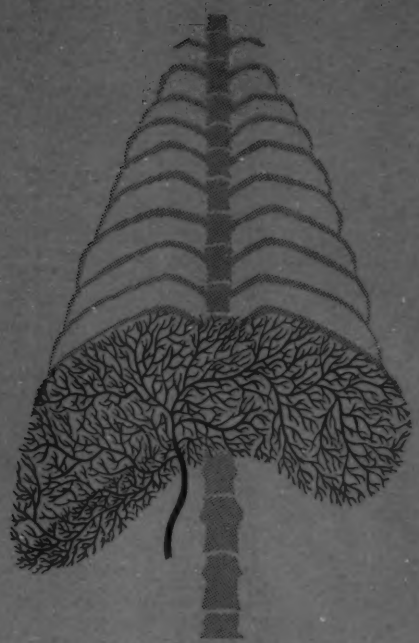

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A.I.B.S. Russian Translations

The AIBS is in the process of expanding its Russian Translations Program extensively. Funds to subsidize translation and publication of important Russian literature in biology have been obtained from the National Science Foundation, as part of a larger program to encourage the exchange of scientific information between the two countries. The following monographs have been scheduled for early publication:

Origins of Angiospermous Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. 68 pgs. Ready now. $3.00 (U.S. & Canada) $3.50 (Foreign)

Essays on the Evolutionary Morphology of Plants. By A. L. Takhtajian. Edited by G. Ledyard Stebbins. Translated by Olga H. Gankin. Ready November 1958. $5.00 (U.S. & Canada) $5.50 (Foreign)

Problems in the Classification of Antagonists of Actinomycetes. By G. F. Gause. Edited by David Gottlieb. Translated by Fritz Danga. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

X-Rays and Plants. By L. P. Breslavets. Ready early 1959. $5.00 (U.S. & Canada) $5.50 (Foreign)

Arachnida. Vol. IV, No. 2. Fauna of the U.S.S.R. By B. I. Pomerantzev. Edited by George Anastos. Translated by Alena Elbl. Ready early 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Arachnoidea. Vol. VI, No. 1. Fauna of the U.S.S.R. By A. A. Zachvatkin. Translated and edited by A. Ratcliffe and A. M. Hughes. Ready spring 1959. $10.00 (U.S. & Canada) $11.00 (Foreign)

Three new Russian journals are being added to the list of four currently translated and published by AIBS:

Soil Science (Pochvovedenie). 12 issues per year. Approx. 1,600 pgs. per year. Will begin with January 1958 issue. Ready January 1959.

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Doklady—Biochemistry Section. 6 issues per year. Approx. 500 pgs. per year. Currently being translated and published by Consultants Bureau, this section of Doklady will now be published by AIBS, beginning with January 1958 issue. Ready September 1958.

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